Investigating human pharmaceutical compounds present in municipal and hospital wastewaters and options for their removal

By

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I certify that this is a true and accurate version of the thesis approved by the examiners.

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ABSTRACT

Pharmaceutical compounds comprise a wide range of substances that are consumed in large quantities by modern societies and are generally released into local sewer networks through excretion. This research aimed to identify the factors affecting the removal efficiencies of these compounds in biological wastewater treatment plants (WWTPs) under different environmental conditions. Of the pharmaceutical compounds selected for this study, the highest influent concentrations measured in municipal wastewater treatment plants (MWWTPs) were for paracetamol, naproxen and bezafibrate (> 1 µg/L), followed by carbamazepine, atenolol, lidocaine, sulfamethoxazole and NACS (< 1 µg/L). In hospital wastewater treatment plants (HWWTPs), the highest concentrations measured were for paracetamol and caffeine (> 10 µg/L), followed by ciprofloxacin and NACS (1–6 µg/L), and finally bezafibrate, carbamazepine, atenolol, lidocaine, clarithromycin and sulfamethoxazole (< 1 µg/L). Antibiotic drugs were detected in HWWTPs, but rarely detected in MWWTPs. In general, the hospital wastewaters contained relatively higher levels of pharmaceuticals than municipal wastewaters.

The removal efficiencies of the pharmaceutical compounds ranged widely. This was found to be related to characteristics and operational parameters of the individual WWTPs. The MWWTPs that utilized long aeration and biomass retention times (HRT,SRT), as evidenced by the occurrence of complete nitrification, were more efficient at removing paracetamol, naproxen, bezafibrate and atenolol, than the non-nitrifying plants with relatively shorter HRT and SRT. HWWTPs that operated under elevated ambient temperatures (> 26°C) achieved higher removal efficiencies (90%) for several compounds, including paracetamol, caffeine, sulfamethoxazole, ciprofloxacin, clarithromycin, NACS, atenolol, carbamazepine and lidocaine. In addition to the elevated ambient temperatures, elevated HRT and SRT and less dilution can lead to increased active biomass and can result in higher removal rates for the pharmaceutical compounds. Overall, the removal efficiencies of pharmaceuticals in WWTPs have been correlated to the type of treatment plant, the plants' operational parameters (HRT, SRT), the climatic conditions (temperature and dilution effect of rainfall) and characteristics of the micropollutants (type and concentration).

Aerobic and anaerobic batch biodegradation experiments were conducted to observe the removal of paracetamol, naproxen, ibuprofen and sulfamethoxazole at various SRTs. The biodegradation rates varied widely ranging from poor, to moderate, to high, depending on the SRT. Paracetamol was highly biodegradable under both aerobic and anaerobic conditions. Sulfamethoxazole was poorly biodegradable under aerobic conditions but highly biodegradable under anaerobic conditions. Relatively slow biodegradation rates were observed for ibuprofen and naproxen under both conditions; longer microbial adaptation periods for these two compounds were probably required. The most important factor affecting the removal of the compounds was the SRT. Therefore, the conclusion was drawn that combining anaerobic and aerobic systems with longer SRT and HRT could bring about significant reductions in the emissions of these contaminants into the environment via WWTPs; this is also a cost-effective option.

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ABBREVIATIONS

%	Percent
<	Less than
>	More than
°C	Temperature as degrees Celcius
µg	Microgram
2,6-DMA	2,6-Dimethylaniline
AC	Activated carbon
AD	Anaerobic digestion
AOPs	Advance oxidation processes
AOX	Absorbable organic halogens
ASP	Activated sludge process
BOD_5	Biochemical oxygen demand
COD	Chemical oxygen demand
CW	Constructed wetland
DDD	Defined daily doses
EC-UWWTD	European Commission Urban Wastewater Treatment Directive
Eff	Effluent
EPA	Environmental Protection Agency
R E	Removal efficiency
ESC	Environment Science Centre
GAC	Granular activated carbon
GCU	Glasgow Caledonian University
H_2O_2	Hydrogen peroxide
HPLC	High liquid performance chromatographic
HRT	Hydraulic retention time
HWW	Hospital wastewater treatment
HWWTP	Hospital wastewater treatment plant
Ibu	Ibuprofen
ICM	Iodinated contrast media
IHWWTP	Iman hospital wastewater treatment plant
Inf	Influent
KSU	King Saud University
LOQ	Limit of quantitation
MASCO	Medical Academic and Scientific Community Organization
MBR	Membrane bioreactor
MEGX	Monoethylglycinexylidide
MENA	Middle Eastern and North African
Mg	Miligram
mL	Milliliter
MMRA	Ministry of Municipalities and Rural Affairs
MOH	Ministry of Health
MOWE	Ministry of Water and Electricity
MPN	Most probable number
MS	Mass spectrometer
MWW	Municipal wastewater

MWWTP	Municipal wastewater treatment plant
Nap	Naproxen
NAS	Nitrifying activated sludge
ng	Nanogram
NGHCAD	National Guidance for Healthcare Wastewater Discharges
NH_4	Ammonium
NHS	National Health Service
NO_2	Nitrite
NO_3	Nitrate
non-NAS	non-nitrifying activated sludge
NSAID	Non-steroidal anti-inflammatory drugs
O_3	Ozone
OECD	Organisation for Economic Co-operation and Development
OH	Hydroxyl radicals
PAC	Powdered activated carbon
Para	Paracetamol
PE	population equivalent
PNEC	Predicted No Effect Concentration
SHWWTP	Salman hospital wastewater treatment plant
SPE	Solid phase extraction
SRT	Solid retention time
SS	Suspended solids
Sulf	Sulfamethoxazole
TF	Trickling filter
TiO_2	Titanium dioxide
TOC	Total organic carbon
TS	Total solids
UK	Unitied Kingdom
UV	Ultraviolet
WWTP	Wastewater treatment plant

CHAPTER 1

General introduction

1.1 Background

Municipal wastewater (MWW) consists of wastewater from households, manufacturing industries and commercial enterprises, and rainwater run-off from roads and other impermeable surfaces (such as roofs and pavements) that drain into sewers (Defra 2012). Untreated wastewater can cause significant damage to the environment. Therefore, different types of wastewater treatment have been designed to remove various contaminants. The treatment of wastewater aims to remove pollutants including pathogens, organic compounds and nutrients. In many countries, MWW and other wastewaters, like hospital wastewater (HWW), are treated together, in centralised sewage treatment plants.

For many years, organic pollutants in surface waters have mainly been measured by analysing the biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (SS) (Cirja *et al.* 2008). However, during the 1970s and 1980s, researchers reported the presence of organic micropollutants, such as human hormones and pharmaceutical compounds, in surface waters (Tabak and Bunch 1970; Tabak, *et al.* 1981). Pharmaceutical compounds, which are consumed in large quantities within modern societies, have since been detected in several different environmental compartments (wastewaters, rivers, lakes, groundwaters, and sediments, etc.) around the world and continue to increase in concentration.

Although there are several different pathways by which human pharmaceutical compounds are discharged into the environment, municipal wastewater treatment plants (WWTPs) are by far the most important source (Figure 1.1).

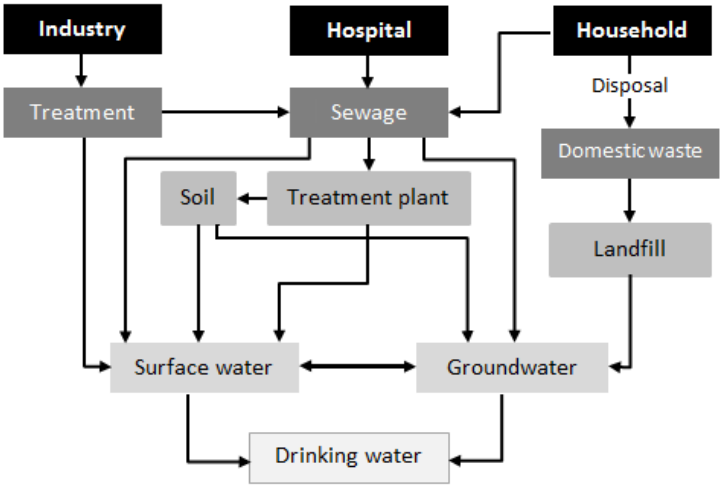

Fig. 1.1: Exposure routes of human pharmaceutical compounds into wastewater and the aquatic environment.

Conventional WWTPs are known to effectively treat carbon, nitrogen and microbial pollutants (macropollutants). However, they also receive a wide variety of micropollutants that may pass through the treatment plant into surface waters without being significantly reduced during treatment. This is due, generally, to the low concentrations of pharmaceutical compounds in effluent and the fact that WWTPs are not normally designed to remove these types of pollutants. Consequently, the continuous input of pharmaceutical compounds and their metabolites into the environment from WWTPs, even at low concentrations, could cause long-term environmental effects and could even affect public health via drinking water. The overall release of these compounds and the effects that they may cause are not fully understood. One exception is endocrine disrupting compounds, which have been extensively assessed and

are known to have negative effects on aquatic organisms. Thus, environmental contamination by pharmaceutical compounds is a worldwide problem that may continue to grow as the potential for pharmaceutical compounds to cause long-term damage within ecosystems begins to be better understood. Public awareness and concern about pharmaceutical compounds in the environment has grown significantly, and has brought the issue to the forefront of water quality management. In particular, there has been an increasing amount of research focusing on risk assessment, aimed at evaluating the effects of these substances in the ecosystems, in light of the increasing consumption of pharmaceutical compounds in modern societies. The European Union has recently taken action, by monitoring micropollutants through several projects that are aimed at quantifying their removal rates during standard and advanced wastewater treatment processes, and their presence in receiving waters. Notable examples of these studies include:

- PILLS project (Pharmaceuticals Input and Elimination from Local Sources, 2007–2012). The PILLS project aims (i) to identify which treatment methods are best suited to reduce pharmaceutical residues and antibiotic resistant bacteria in wastewater, (ii) to gain more knowledge about the circumstances under which local treatment, for example at hospitals, is reasonable and (iii) to increase awareness of this problem across Europe (PILLS project 2012).

- BIOTREAT (Bio-treatment of drinking water resources polluted by pesticides, pharmaceuticals and other micropollutants). BIOTREAT is a

European Union (EU) project initiated on 1 January 2011 aiming to develop new technologies for bioremediation of drinking water resources contaminated with micropollutants, such as pesticides and pharmaceuticals (Biotreat 2013).

- “Measures to reduce the input of micropollutants into water bodies” (2012–2014). The purpose of this project was to develop appropriate measures to reduce the input of micropollutants discharged by the municipal wastewater treatment plants and sewer systems into water bodies (KIT 2013).

In addition, the European Union has taken legislative action on micropollutants, with Directive 2013/39/EU of the European Parliament and of the Council (European Commission 2014), in order to minimise the risks to the environment.

1.2 Aim of this research

The overall aim of this research was to identify the factors affecting the removal efficiencies of pharmaceutical compounds in biological WWTPs under different environmental conditions, in order to propose guidelines for their reduction in the aquatic environment.

1.3 Objectives

The objectives of the research include the following:

- Identify a common point source of pharmaceutical compounds in MWWs.

- Identify the important pharmaceutical compounds that are released by the point source.
- Identify the factors that affect the removal efficiency of those pharmaceutical compounds under different environmental conditions.
- Assess the fate of pharmaceuticals during different biological treatment systems.
- Recommend feasible methodologies for the treatment of common human pharmaceutical compounds under various climatic conditions.

Two main field studies were carried out at WWTPs in Saudi Arabia and the UK and an additional laboratory study was conducted (biodegradation experiments) to address the objectives of the research.

1.4 Organisation

This thesis is divided into eight chapters, including this introduction and the conclusions. Furthermore, each chapter is subdivided into further subsections.

This chapter (**Chapter 1**) has presented an overview of the issue that this research aims to address and the organisation of this work.

Chapter 2 consists of a literature review of HWWs, identified to be the main point source of pharmaceutical compounds in MWWs. The chapter includes an analysis of the sources of HWW, characterisation of HWW (the consumption of water and pharmaceutical compounds in hospitals) and the known fates of pharmaceutical compounds during different wastewater treatment processes.

The differences between macropollutants and micropollutants in wastewater are also discussed.

In **Chapter 3** the methodologies employed for the research are presented. This includes the rationale for selecting (i) the pharmaceutical compounds to monitor in the WWTPs, and (ii) the locations and types of WWTPs. In addition, details of the sampling and analysis methods for the wastewaters are provided. The methodologies used in laboratory biodegradation experiments have also been included in this chapter.

Chapter 4 reviews the current hospital wastewater management practices in the UK and Saudi Arabia, the countries used as case studies in this research. The review presents the collection, treatment and disposal of hospital wastewaters in these countries.

Chapter 5 presents and discusses the results of the field studies conducted at different wastewater treatment plants across the UK and Saudi Arabia. These studies investigated the average concentrations of conventional pollutants and pharmaceutical compounds in the selected wastewater treatment plants. The potential influence of the operational parameters on removal efficiencies is discussed.

Chapter 6 presents the results of the laboratory biodegradation experiments, which consider the potential biodegradation of selected pharmaceutical compounds under aerobic and anaerobic conditions, in order to assess and discuss the fate of pharmaceuticals under these important conditions.

Chapter 7 proposes management practices for pharmaceutical compounds in WWTPs, in tropical and temperate climates. Finally, **Chapter 8** presents the general conclusions of this research and recommendations for future research.

CHAPTER 2

Pharmaceuticals in hospital wastewater

Sources, characterisation and fate

2.1 Introduction

Pharmaceutical compounds constitute a wide group of compounds that are largely used for the treatment of health conditions and protection of humans and other animals from disease. When they are released into the aquatic ecosystems, they may have an adverse effect on the organisms inhabiting those ecosystems. These pollutants, in general, originate from urban environments, are collected in wastewater collection systems and may finally reach the aquatic natural environment in discharged effluent. Until recently, these compounds have not been of a major concern with regard to their environmental effects.

Studies have shown that hospitals are a major point source of pharmaceutical compounds to wastewater treated in wastewater treatment plants (WWTPs) (Helwig *et al.* 2013). Hospital wastewater (HWW) contains various hazardous and toxic materials, such as microbiological pathogens (e.g. antibiotic resistant bacteria and viruses), hazardous chemical compounds, disinfectants, pharmaceutical compounds, and radioactive isotopes, among others (Pauwels and Verstraete 2006; Verlicchi *et al.* 2010a; Verlicchi *et al.* 2012b: Permatasari and Mangkoedihardjo 2012). HWW is generally connected to urban sewer systems, so municipal wastewaters (MWW) and HWW are usually co-treated in conventional WWTPs (Alder *et al.* 2006). This is where the problem really begins, as municipal wastewater treatment plants are not designed to remove

medical or pharmaceuticals waste. The scientific community recommends the pre-treatment of HWWs, to enhance the degradation of micropollutants (Altin *et al.* 2003; Pauwels and Verstraete 2006), before discharge into municipal wastewater collection and treatment systems. Hospital management strategies have therefore become a subject of lively discussion in the scientific community, which has recently started to evaluate the contributions of HWWs to MWWs, in terms of the micropollutant loads at local point sources (WWTPs) into the environment (Verlicchi *et al.* 2012b).

2.2 Water consumption in hospitals

Hospitals are amongst the largest water consumers in a community. They require significant amounts of water for various uses and applications. Hospitals typically consume water at between 500 and 1200 L/bed/day (Emmanuel *et al*. 2005). The water consumption of an individual hospital depends on several factors, including number of beds, hospital age, accessibility to water, general services present inside the hospital (e.g., kitchen, laundry and air conditioning) and the number of wards and units (Verlicchi *et al.* 2010a,b). In addition, the institution's management policies and awareness in managing the hospital and safeguarding the environment, climate, cultural and geographical factors are important (Verlicchi *et al.* 2010a, b).

Water consumption varies from country to country, during different seasons, and even during the day. For example, the average water consumption in the USA and France is 968 and 750 L/bed/day, respectively; for developing countries it is just 500 L/bed/day (Emmanuel *et al*. 2005). In terms of the effect

of season, higher average consumption values were observed during the summer time in multiple studies (Joss *et al.* 2005; Mohee 2005; Boillot *et al.* 2008). During the day, flow rates are increased up to 20% above average between 8 a.m. and 4 p.m., and are 30% below average between 1 a.m. and 8 a.m. (Verlicchi *et al.* 2010a). Sanitation consumes the most water out of the major hospital services, according to an analysis of seven European hospitals, ranging from 130 to 500 beds, with water consumption rates between 56 and 549 million L/year (Figure 2.1) (ESC 2013).

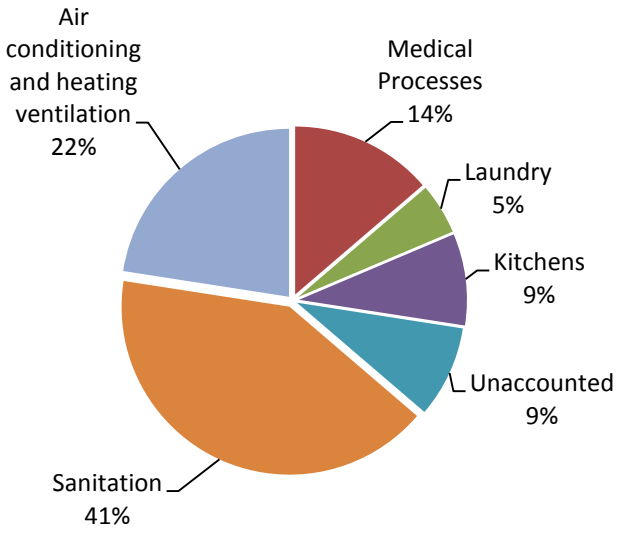

Fig. 2.1: Water consumption in hospitals (ESC 2013)

The consumption of water in hospitals is expected to increase as a result of the development of medical services and health care products. Alongside this increase in the consumption of water, hospitals are expected to generate increasingly significant amounts of wastewater. Mesdaghinia *et al.* (2009) compared the average water consumption per bed with the average wastewater production in eight hospitals and their results indicated that 75–85% of the total fresh water consumed in hospitals becomes wastewater (Figure 2.2).

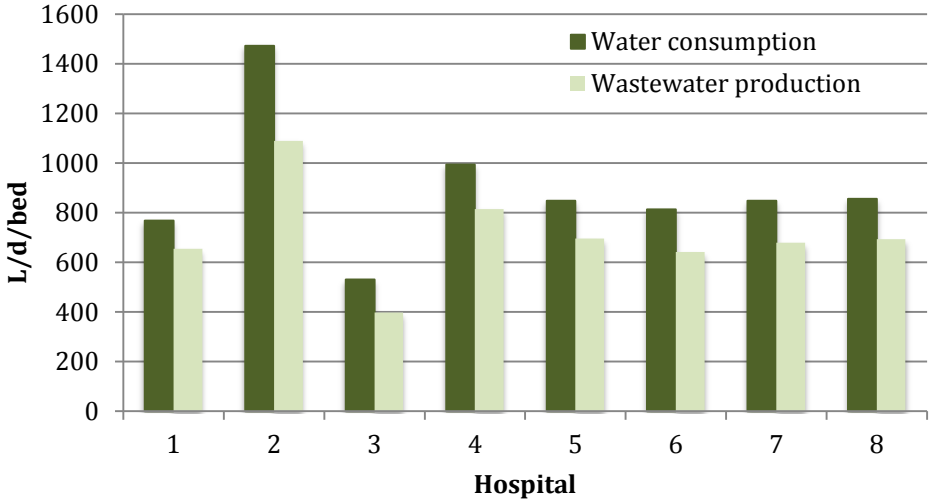

Fig. 2.2: The average water consumption and wastewater production per bed in eight hospitals located in Iran (Mesdaghinia *et al*. 2009)

2.3 Pharmaceutical consumption and hospital contribution

The consumption of pharmaceutical compounds has increased significantly since the 1950s due to factors including population growth, the development of medical science and the emergence of diseases, particularly infectious diseases (Le Corre *et al.* 2012). The consumption rate of common pharmaceutical compounds (antidiabetics and antidepressants) has continued to increase during the last decade in several countries (Figure 2.3) (OECD 2014). Country-wide data on the consumption of pharmaceutical compounds in hospitals, especially for inpatients, remains limited. Helwig *et al.* (2013) reported the consumption of selected pharmaceutical compounds in hospitals across different European countries, in terms of the annual totals of the prescribed medicine at each hospital (Table 2.1).

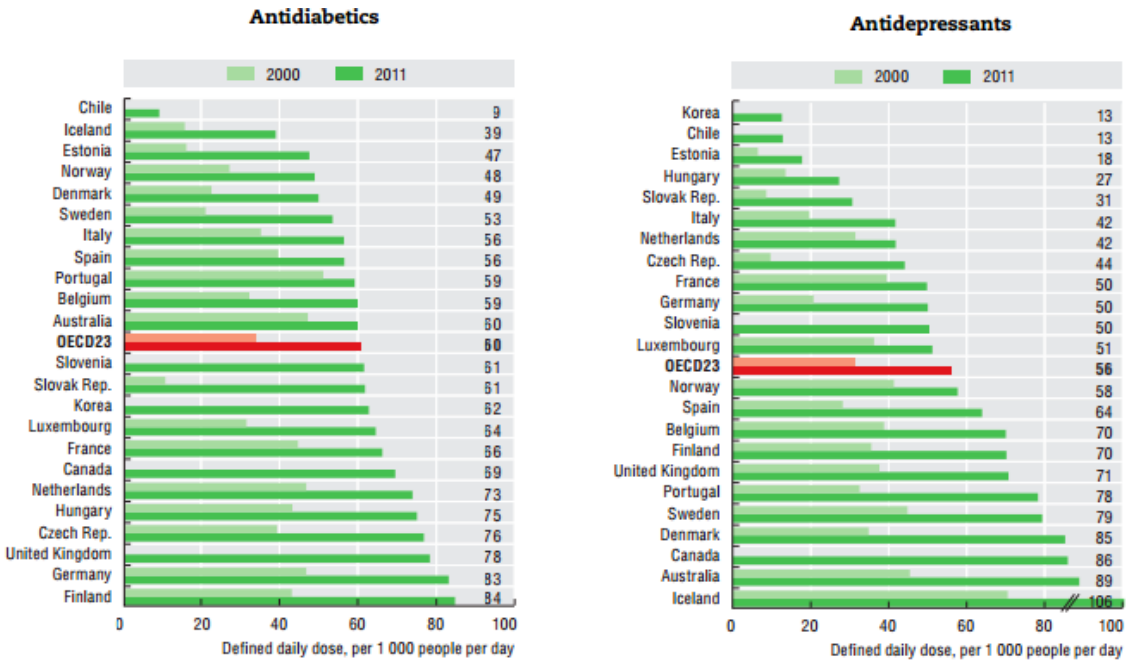

Fig. 2.3: The consumption of antidiabetics and antidepressants in 2000 and 2011 for different countries (OECD 2014).

Table 2.1: The average annual consumption of selected pharmaceutical compounds in hospitals across different European countries (Helwig *et al.* 2013)

	Hospital pharmaceutical consumption (g/bed/year)					
Country[1]	DE	LU	FR	NL	UK[2]	UK[3]
Year	2011	2010	2010	Mean	2011	2011
No. of beds	560	360	863	1076	265	318
Diclofenac	7.07	1.62	1.93	0.50	3.26	3.98
Naproxen	0.00	8.73	2.18	0.07	3.98	1.85
Carbamazepine	1.64	1.00	2.91	0.00	4.34	4.49
Atenolol	0.27	0.15	0.64	0.00	2.20	1.59
Bezafibrate	0.84	0.00	0.00	0.01	0.78	0.41
Lidocaine	0.00	2.21	46.95	2.36	17.21	8.45
Amoxicillin	22.73	92.52	67.05	47.49	72.26	125.37
Ciprofloxacin	9.61	16.50	21.80	8.96	27.31	24.19
Clarithromycin	3.82	5.47	0.85	0.40	24.75	18.04
Sulfamethoxazole	3.32	0.33	13.39	5.37	0.00	15.07
Erythromycin	2.18	0.55	2.93	1.45	2.12	2.41
Diatrizoate	67.06	0.00	181.37	3.89	0.00	29.45
Iopamidol	13.49	0.00	4.36	0.00	687.67	9.63
Iopromide	0.00	0.00	0	248.42	4.26	0.00
Cyclophosphamide	0.31	0.48	1.79	1.94	0.00	8.45
Ifosfamide	0.10	0.28	0.71	0.03	0.18	0.00

[1]DE (Germany), LU (Luxemburg), FR (France), NL (the Netherlands) and UK (Scotland); [2]Rural UK hospital; [3]Urban UK hospital.

The investigation by Helwig *et al.* (2013) indicated that consumption (by weight) of the pharmaceutical compounds was highest for contrast media (for single compounds up to 981 g/bed/annum), while generally there were also relatively high consumption figures for antibiotics (typically 5–25 g/bed/annum).

Vogler *et al.* (2010) indicated that the most commonly consumed drugs in European hospitals are paracetamol, furosemide, acetylsalicylic acid, epoetin beta and albumin. Table 2.2 shows the share percentages of the total volume of selected drugs that were used in hospitals in Germany, in 1998 and 2001, and demonstrates that all of the contrast media and cytostatic drugs examined, as well as ampicillin, penicillin G and vancomycin (of the antibiotics examined), were mainly administered in hospitals (Alder *et al.* 2006). Antibiotic groups are among the most common drugs consumed worldwide. The worldwide antibiotic market consumption was previously estimated to be between 100,000 and 200,000 tons annually, of which approximately 25% was used in hospitals (Kümmerer 2003; Wise 2002). The consumption of antibiotics increased by 36% between 2000 and 2010 (Van Boeckel *et al.* 2014).

Once pharmaceuticals have been administered to patients, the dosages are excreted as either parent compounds or metabolites via urine and faeces into wastewater treatment plants. However, a significant proportion of drugs dispensed in hospitals are issued to patients but not excreted within the hospital environment (Helwig *et al.* 2013). Overall, the consumption and application of pharmaceutical compounds in a hospital vary over the year and from country to country (Schuster *et al.* 2008).

Table 2.2: Hospital usage of selected drugs as a percentage (%) of total use in Germany (Alder *et al.* 2006).

Drugs	Compound	1998	2001
Antibiotics	Amoxicillin	12	11
	Ampicillin		86
	Ciprofloxacin	34	31
	Metronidazol	38	50
	Penicillin g	93	94
	Sulfamethoxazole	13	12
	Vancomycin	92	94
	Clarithromycin	9	13
Contrast media	Diatriaoate	98	98
	Iomeprol	100	100
	Iopamidol	96	97
	Iopromide	100	100
Cytostatic	Cyclophosphamide	67	67
	Ifosfamide	95	97

The contribution of pharmaceutical compounds from HWWs to the receiving WWTPs vary widely, depending on the different groups of pharmaceuticals, and from hospital to hospital (Verlicchi *et al.* 2010a). Helwig *et al.* (2013) reported the concentrations of some antibiotics from different European hospital wastewaters compared to MWWTPs to be up to 154% (ciprofloxacin), 59% (clarithromycin), 53% (sulfamethoxazole) and 82% (erythromycin) of the concentrations found. Another study found that the concentration of ciprofloxacin in HWW was 272% higher than the local MWWTP in Norway (Thomas *et al.* 2007). Antibiotic groups are highly toxic to organisms in the environment, especially algae and bacteria (Pauwels and Verstraete 2006; Watkinso *et al.* 2009). Some antibiotics, used in hospitals, are designed to cause DNA damage to bacteria or eukaryotic cells (Kümmerer *et al.* 2000). The

concentrations levels of antibiotics in HWWs were reported between 4 and 100 times greater than their respective concentrations in domestic wastewaters (Joss *et al.* 2006).

Santos *et al.* (2013) reported that non-steroidal anti-inflammatory drugs (NSAIDs), analgesics and antibiotics were the largest contributors to the total load of pharmaceuticals at WWTPs that originated from HWWs, corresponding to 51%, 41% and 32%, respectively. Sources of the X-ray contrast agent (iopromide and iopamidol) in HWW were found to contribute up to 100% to the final WWTP load (Helwig *et al.* 2013).

In general, the consumption of pharmaceuticals and relative hospital contribution to wastewater vary widely amongst different therapeutic groups, hospitals and countries depending on lifestyle, hospital size, legislation, economy, types of prevalent diseases and population size (Santos *et al.* 2013; Helwig *et al*. 2013; Kümmerer and Schuster 2008).

2.4 Disposal of pharmaceutical compounds in hospitals

Pharmaceutical compounds from hospitals are released into MWWs via two main pathways: excretion from patients or through unsuitable disposal (i.e. disposed of down sinks or toilets). For the disposal of unwanted drugs, there are generally standard procedures that hospitals should use (WHO 2014). However, in some instances, abuse of these procedures by hospital staff and/or patients occurs during the disposal of unwanted drugs (Kallaos *et al.* 2007). For example, unwanted pharmaceutical compounds may be illegally disposed of into the sewage system or as solid waste. Disposal into the sewage system

contributes to raising the concentrations of pharmaceutical compounds in HWWs and eventually MWWs. Kallaos *et al.* (2007) investigated the disposal methods of unwanted pharmaceuticals in different institutions, including hospitals, nursing homes and pharmacies. Their study showed that the disposal of unwanted drugs by direct disposal into the drainage system was not a common disposal method (Figure 2.4).

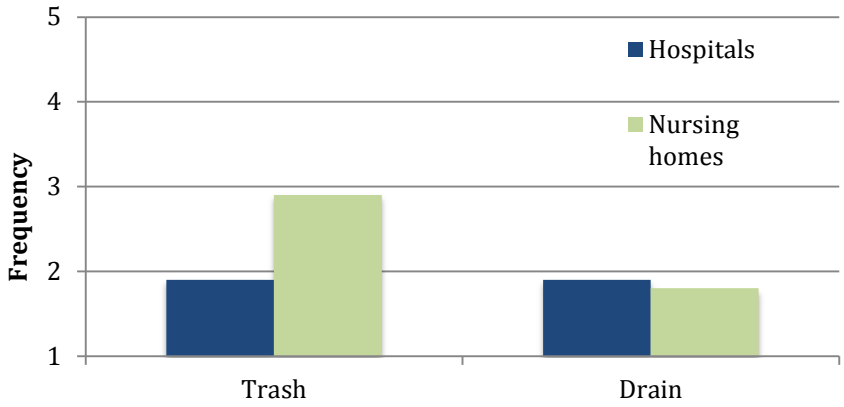

Fig. 2.4: The average frequency scores of the disposal methods for unwanted drugs used in hospitals and nursing homes in Santa Barbara, USA. Trash = solid waste; Drain = disposal to drainage system; and frequency scores represent a gradient from never (1) to very frequently (5) (Kallaos *et al.* 2007).

2.5 Hospital wastewater sources

Hospital wastewater contains various hazardous compounds including microbiological pathogens, hazardous chemical compounds, disinfectants, pharmaceutical compounds and radioactive isotopes, as discussed in Section 2.1. It is important to understand the sources within hospitals that contribute to the generation of hazardous pollutants. If these are known, the hazardous and/or toxic compounds in HWW can be predicted and effectively managed at their source. An effective HWW management system requires knowledge of the source points, and the adverse impacts the compounds have on the aquatic

environment. Although it is rather difficult to estimate and classify the units of HWW discharged, this section describes the sources of wastewater from different hospital units. A previous study reported that HWW comes from three essential units of the hospital (Verlicchi *et al.* 2010a):

1. General units: such as the laundry, kitchen, and heating and cooling systems.
2. Medical units: such as the laboratories, radiology units and haemodialysis units.
3. Patient ward units: such as general medicine, surgery, specialities, haemodialysis, and intensive care, among others.

The wastewater that comes from these various units differs in both quality and quantity. The wastewater that is generated from the laundry and kitchen units is generally similar quality to urban wastewater and is classified as domestic wastewater (Verlicchi *et al*. 2010a). The general units usually produce approximately 40% of the total HWW and these units do not normally pose serious challenges to the treatment processes or include hazardous compounds.

On the other hand, wastewater that comes from medical units and patient wards is likely to be loaded with multiple pollutants that are toxic and hazardous. The Medical Academic and Scientific Community Organization (MASCO 1996) classified the sources of pollutants from hospitals into four categories, three of which constitute 'medical units' (Table 2.3); each category is a source of different types of pollutants. The major source of pharmaceutical compounds in WW is excretion by patients (Jjemba 2006). Patients excrete these compounds

via urine or faeces (as discussed in Section 2.4) as parent compounds or metabolites (Langford and Thomas 2009).

Table 2.3: Type and source of pollutants from hospital units (MASCO 1996)

Sources	Pollutants	Comparison of BOD_5[1] and COD[2] with domestic waste water
Clinical laboratory	Heavy metals, organic chemicals, blood products and body fluids, phosphates, oil, grease, particulate materials, buffers, and dilute mineral acids/bases.	BOD_5 and COD values are higher than domestic wastewater values.
Research laboratory	Oxidizers, radio nuclides, proteins, oil, grease, heavy metals, organic solvents, blood products and body fluids, formaldehyde, phosphates, detergents, and photographic imaging chemicals.	BOD_5 and COD values are lower than clinical laboratory, but above average domestic wastewater
Medical waste incinerators	Low organic materials and oxidizers, high particulate materials and heavy metals.	BOD_5 and COD values are lower than domestic wastewater
Hospital laundry	Fats, oil, grease, detergents, proteins, and oxidizers	BOD_5 and COD values are usually in the normal range for domestic wastewater

[1]Biochemical oxygen demand; [2]Chemical oxygen demand.

2.6 Characteristics of hospital wastewater

It is useful to understand the characteristics and quantities of HWW being generated, in order to optimise wastewater treatment processes. As already noted (Section 2.1), hospital wastewater contains a wide variety of pollutants including organic and inorganic pollutants. Usually, organic pollutants in HWWs are composed of natural organic matter, soluble microbial products, harmful chemicals and by-products that are known as dissolved organic matter (>0.45µm) (Shon *et al.* 2006). These pollutants, either macropollutants or

micropollutants, play an important role in the composition and classification of HWWs.

2.6.1. Macropollutants

Macropollutants (i.e. conventional pollutants) are quantitatively estimated, mainly through the measurement of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), suspended solids (SS), and total coliforms, among other measures. A comparison of macropollutants between HWWs (hospital sizes 60–900 beds) and MWWs showed that macropollutants in HWWs are generally higher than in MWWs (Table 2.4) (Verlicchi *et al.* 2010b). In particular, conventional parameters, such as COD and BOD_5 were higher; these are the most common indicators used to measure the amount of organic macropollutants in waste waters.

Table 2.4: Comparison of average concentrations of macropollutants between HWW and MWW (Verlicchi *et al.* 2010b).

Parameter	Unit	HWW	MWW
pH	-	7.0–9.0	7.5–8.5
Redox potential	mV	850–1000	420–1340
TKN[1]	mg/L	5–100	20–70
Total phosphorus	mg/L	0.2–13	4–10
Fat and oil	mg/L	5–60	50–150
Chlorides	mg/L	65–400	30–100
Total surfactants	mg/L	3–7.2	4–8
Escherichia coli	MPN/100mL	10^3–10^6	10^6–10^7
Faecal coliforms	MPN/100mL	10^3–10^7	10^6–10^8
Total coliforms	MPN/100mL	10^5–10^8	10^7–10^{10}
BOD_5[2]	mg/L	150–603	100–400
COD[3]	mg/L	450–2300	500–600
SS[4]	mg/L	120–400	120–350

[1]Total kjeldahl nitrogen; [2]biochemical oxygen demand; [3]chemical oxygen demand; [4]susepended solids

Several studies have investigated BOD_5 and COD concentrations in raw HWWs from different countries (Table 2.5); BOD_5 values ranged from 100 to 1545 mg/L while COD values ranged from 337 to 2590 mg/L. The levels of conventional macropollutants in HWWs vary from country to country, and are also related to the size of the hospital and the type of services supplied. In general, the concentrations of organic macropollutants in HWW are higher than those in MWW but do not pose a significantly different challenge compared to MWW in wastewater treatment processes.

Table 2.5: The average concentrations of BOD_5 and COD (mg/L) in raw hospital wastewater from different countries.

Country	BOD_5	COD	Reference
Thailand	410	1350	Kajitvichyanukul *et al.* 2006
Italy	240	480	Verlicchi *et al.* 2010a
Iran	245	592	Sarafraz *et al.* 2006
Iran	412	814	Mahvi *et al.* 2009
Egypt	210	570	El-Gawad *et al.* 2011
Turkey	147	337	Altin *et al.* 2003
Iran	444	789	Mesdaghinia 2009
Greece	348	527	Nasr *et al.* 2008
France	1545	2590	Emmanuel *et al.* 2005
France	603	855	Emmanuel *et al.* 2002
Brazil	100	380	Chagas *et al*. 2011
Spain	—	490	Cruz-Morato *et al.* 2014

2.6.2. Micropollutants

Hospital wastewater also contains several varieties of dissolved chemical substances (< 0.45 µm), including micropollutants, which are generally present at low concentrations (between µg/L and ng/L). These micropollutants are also considered to be emerging contaminants and include pharmaceutical compounds, hormones, fragrances, disinfectants, halogens, heavy metals and

other organic and inorganic compounds (Verlicchi *et al.* 2012a; Verlicchi *et al.* 2010b). Many new pharmaceutical compounds and health care products are continuously being introduced into the market, due to new legislation, healthcare improvements and the emergence of new diseases. This section focuses on pharmaceutical compounds as environmental contaminants. These compounds, used largely in healthcare are mainly organic compounds. There are many different classes of pharmaceutical compounds that are commonly used in hospitals (Table 2.6) (Verlicchi *et al.* 2010b).

Table 2.6: Main classes of pharmaceutical compounds that are consumed in hospitals (Verlicchi *et al.* 2010b).

Class	**Example**
Antibiotics	Sulfamethoxazole, ciprofloxacin, coprofloxacin, chlortetracycline, erythromycin, doxycycline, lincomycin, norfloxacin, ofloxacin, oxytetracycline, penicillin, cefazolin,, tetracycline, trimethoprim
Analgesics and anti-inflammatories	Ibuprofen, naproxen, paracetamol, diclofenac, indomethacin, ketoprofen, mefenamic acid, propyphenazone, salycilic acid, codeine, dipyrone.
Cytostatics	5-Fluorouracil, ifosfamide
Anaesthetics	Propofo, lidocainel
Disinfectants	Triclosan, glutaraldehyde
Iodized contrast media (ICM)	Iopromide, iopamidol
Psychiatric drugs, antidepressants, anticonvulsants	Carbamazepine, gabapentin, phenytoin, valproic acid
Antihistamines	Ranitidine, cimetidine
Antihypertensive	Diltiazem
Antidiabetics	Glibenclamide
B-blockers	Atenolol, metroprolol, propranolol, solatolol
Hormones	17 b-Estradiol, estriol, estrone, ethinylestradiol
Lipid regulators	Atorvastatina, bezafibrate, clofibric acid, gemfibrozil, pravastatin
Diuretics	Furosemide, hydrochlorotiazide
Stimulants	Caffeine
Heavy metals	Platinum, mercury
Musk and fragrance	Tonalide, galoxolide

Most of the pharmaceutical compounds in Table 2.6 are administered to patients for oral intake or via intravenous application. Either way, they are eventually excreted by patients into the sewer system as metabolites or un-metabolised forms. Some pharmaceutical compounds may be readily metabolised, moderately metabolised, poorly metabolised, or remain un-metabolized after consumption. Parent pharmaceuticals and their metabolites differ in both their pharmacological and toxicological properties. Although pharmaceutical compounds are usually present in relatively small concentrations in wastewater, some of them are more stable than macropollutants and cannot be quickly broken down during wastewater treatment processes. Therefore, they pose an additional challenge for the wastewater treatment processes and can pass into environmental systems, where they can cause negative effects (Fent 2008).

Hospital wastewaters are significant sources of antibiotics, anaesthetics, anti-cancer drugs, anti-inflammatories, disinfectants, heavy metals, adsorbable organic halogens (AOX), contrast media and cytostatic agents (Kümmerer 2001). For example, in a study of a hospital WWTP in Germany, high concentrations of antibiotics (particularly ciprofloxacin and erythromycin) were detected in both the influent and effluent (Ohlsen *et al.* 2003). Further, primary DNA damage in bacteria within HWWs was correlated strongly with ciprofloxacin concentration (0.7–124.5 µg/L) (Hartmann *et al.* 1999). This study confirmed the growing evidence that active pharmaceutical compounds from hospitals, in this case, antibiotics, are being distributed into the aquatic environment and have negative effects on organisms.

Iodinated contrast media (ICM) are also important pharmaceutical compounds within hospitals. The worldwide consumption of ICM was previously measured to be approximately 3.5×10^6 kg/year (Perez and Barcelo 2007b). Oleksy-Frenzel *et al.* (2000) detected high concentrations, of up to 130 µg/L, of organic iodine compounds in MWWTP in Berlin and up to 10 mg/L in a HWW. These compounds are not readily biodegradable during conventional wastewater treatment processes (Pauwels and Verstraete 2006).

One major concern regarding the input of pharmaceutical compounds into the environment is their genotoxicity. For example, hormonal drugs can disturb the hormonal balance and cause hermaphroditism in fish (Purdom 1994). The endocrine disrupting residues may not just be a problem for aquatic organisms, but may also affect the human population (Casals-Casas & Desvergne 2011; Yu *et al.* 2013). Polluted effluent discharged to freshwater ecosystems can eventually reach oceanic ecosystems, potentially endangering marine wildlife; thus the pollution problem caused by chemical contaminants in HWWs is a global issue.

2.7 Fate of pharmaceuticals in common biological wastewater treatment systems

2.7.1 Activated sludge

The activated sludge process (ASP) is the most popular biological treatment process employed particularly in municipal WWTPs throughout the world. Many ASP plants operate under aerobic condition. The majority of published data show a significant variation in the removal efficiencies of pharmaceutical

compounds by ASP ranging from high (e.g., paracetamol and ibuprofen) to poor (e.g., carbamazepine) (Kim *et al.* 2005; Alvarez *et al.* 2002; Marquez *et al.* 2011). The significant removal reported refers to biodegradation or sorption on solids (Barret *et al.* 2010). The levels of biodegradation in ASP have been reported to be affected by operational parameters (McAdam *et al.* 2010; Sahar *et al.* 2011) (see Section 2.8). There is evidence that some parameters such as solid retention time (SRT), hydraulic retention time (HRT) and temperature affect removal efficiencies (Fernandez-Fontaina *et al.* 2012; Verlicchi *et al.* 2012a), which might explain the variation in removal efficiencies reported in the literature. These factors govern both reaction time and loading (Fernandez-Fontaina *et al.* 2012), thus affecting biomass activity and concentration. Increasing SRT increases the diversity of the consortia of bacteria present in a treatment plant allowing the growth of the slower growing organisms that can only colonise the treatment plant at high sludge ages (Koh *et al.* 2009). Therefore, optimum conditions for bacterial activity may lead to increase biodegradation of micropollutants during treatment processes.

The ASP can be applied in large and small volumes of wastewater. A disadvantage of the ASP is that the removal of pharmaceutical compounds varies between compounds, generating large quantities of solid sludge that requires additional treatment and cost for its disposal (Huber *et al*. 2014).

2.7.2 Trickling filter

Trickling filters (TF) are used to remove pollutants from wastewater as a part of an aerobic process, utilizing microorganisms attached to a medium. Wastewater

is generally spread over the media surface (rocks or plastic) using a rotating arm (Pearce and Edwards 2011). The removal efficiency of TF processes for micropollutants has been found to be less than 70% removal of 55 pharmaceutical compounds studied. In comparison, the WWTP utilising activated sludge treatment gave a much higher removal efficiency of over 85% (Kasprzyk-Hordern *et al*. 2009b). Moreover, trickling filters, in comparison with other processes, are not the most efficient systems for the removal of pharmaceutical compounds (Bartelt-Hunt *et al.* 2009).

2.7.3 Membrane bioreactors

Membrane bioreactors (MBR) consist of a structure that integrates biological treatment with a membrane filtration system in a single procedure (Melin *et al.* 2006). They were first reported in 1969 (Ng and Kim 2007). Membrane bioreactors can be operated at different SRTs (Verlicchi *et al.* 2010a), but the most important advantage of MBRs is that they can be operated at higher biomass concentrations; this results in smaller WWTP sizes and produces less sludge than the more conventional ASP. Several studies have examined the effectiveness of MBRs in the removal of pharmaceutical compounds (Clara *et al*. 2005b; Radjenovic *et al*. 2009; Kim *et al*. 2014). Kim *et al*. (2014) investigated the removal of 99 pharmaceutical compounds through a WWTP that employed an aerobic MBR; they found that the removal of compounds varied from 34–99%. Although many studies (Verlicchi *et al.* 2010a; Radjenovic *et al*. 2009) have indicated that MBRs can remove several pharmaceutical compounds, no significant differences in the removal efficiency of certain

compounds (e.g., ibuprofen, triclosan and caffeine) were found between the MBR and conventional ASP by Oppenheimer *et al.* (2007). Clara *et al.* (2005b) and Radjenovic *et al*. (2009) also compared the effluent concentrations and removal rates for different pharmaceutical compounds in a conventional ASP and a MBR. Their results suggested that there were only slight differences, in favour of the MBR technology, in the removal efficiencies of pharmaceutical compounds. Moreover, for compounds that are persistent when treated with conventional ASP (such as carbamazepine), poor removal efficiencies were also found with MBR treatments (Clara *et al*. 2005b).

As with ASP, the removal potential of MBRs also depends on factors such as the SRT. There are also some disadvantages of MBRs, which include higher capital costs, high costs of operation and maintenance, and high energy requirements of the air blowers and pumps (Clara *et al.* 2005b).

2.7.4 Anaerobic processes

Some pharmaceutical compounds can be removed from wastewater by their adsorption onto solids. Later, if the sludge is inadequately treated, the pharmaceutical compounds can enter the aquatic environment via the application of sludge to land for fertilization or through landfilling. Since 1999, sludge intended for land application in some countries has required advanced treatment through anaerobic digestion (Akunna and Bartie 2014). Anaerobic digestion processes are used to treat 75% of sludge that is generated in the UK, with the degree of treatment depending on the intended final use (Defra 2012).

Some studies have investigated the biodegradation efficiency of some pharmaceutical compounds under anaerobic processes (Carballa *et al.* 2007; Mussan *et al.* 2010). The reported biodegradation efficiency of pharmaceutical compounds has varied from no elimination to high elimination. For example, Carballa *et al.* (2007) observed significant elimination rates for some antibiotics and natural estrogens, while there was no elimination of carbamazepine. Mussan *et al.* (2010) investigated the fate of six pharmaceutical compounds (17α-ethynylestradiol, acetaminophen, acetylsalicylic acid, ibuprofen, metoprolol tartrate, and progesterone) during anaerobic digestion and only found a significant biodegradation potential for acetylsalicylic acid. The efficiency of anaerobic biodegradation is also affected by SRT (Lee *et al.* 2011). Anaerobic digestion uses less energy, and in fact produces energy in the form of methane generation. It is also a relatively easy process to operate, especially in hot climates.

2.8 Factors effecting removal of pharmaceutical compounds in biological treatment systems

The removal of organic compounds during biological treatments is influenced by many factors, such as the chemical and biological properties of the compound, wastewater characteristics, operational conditions, and the treatment technology used (Verlicchi *et al*. 2012a).

2.8.1 Temperature

Temperature conditions during biological wastewater treatment processes can significantly affect the microbial activity and growth (LaPara *et al*. 2000; Vieno *et*

al. 2005; Massmann *et al*. 2006). Akratos and Tsihrintzis (2007) found a positive linear correlation between temperature and the removal of COD, BOD_5 and nutrients. In terms of pharmaceutical compounds, seasonal variations in the removal efficiency of some compounds in WWTPs were reported in Europe and North America, with lower removal efficiencies observed in winter at low temperatures (Heberer 2002; Kolpin *et al.* 2002; Metcalfe *et al.* 2003; Miao *et al.* 2005).

Castiglioni *et al.* (2006) reported that summer temperatures (average 18.6°C) had a strong positive effect on the removal efficiency of most compounds, compared to winter temperatures (average 9.7°C) (Table 2.7).

Table 2.7: Average removal efficiencies of selected pharmaceutical compounds in winter and summer (Castiglioni *et al.* 2006).

Compound	**Average removal efficiency (%)**	
	Winter	Summer
Atenolol	10	55
Bezafibrate	15	87
Enalapril	18	100
Furosemide	8	54
Ibuprofen	38	93
Ranitidine	39	84
Sulfamethoxazole	17	71
Amoxilline	75	100
Ciprofloxacin	60	60
Hydrochlorothiazide	30	30
Ofloxacine	50	50
Carbamazepine	0	0
Clarithromycin	0	0
Erythromycin	0	0
Salbutamol	0	0

These studies clearly demonstrate that higher temperatures improve the removal efficiencies of many compounds. However, little is known about the

fates of pharmaceutical compounds in tropical climates, where there are elevated temperatures year-round. Based on the reported effects of temperature on removal efficiencies of these compounds, it is expected that their removal efficiencies will also be relatively high in tropical temperatures. The concentrations of these compounds may, however, be higher in wastewaters in these countries due to higher water loss by evaporation. More information is therefore needed on the occurrence and fates of these compounds under tropical climatic conditions.

2.8.2 Solids retention time

Solids retention time (SRT) or sludge age is a parameter that is commonly optimised in the design of WWTPs, and indicates the mean residence time of microorganisms in the reactor. The SRT affects the performance of the treatment process, the aeration tank volume, the amount of sludge produced and the oxygen requirements (Jelić *et al.* 2012). Longer SRTs have been shown to influence and improve the elimination of pharmaceutical compounds during biological treatment (Falas *et al.* 2012; Koh *et al.* 2009).

Clara *et al.* (2004) reported that longer SRTs (10 days) of ASP influenced the biodegradation efficiency of some pharmaceutical compounds, such as ibuprofen, bezafibrate, and oestrogens. A positive correlation was found between the removal rates of some compounds (ketoprofen and naproxen) and the SRT, up to 10 days, during an ASP treatment (Falas *et al.* 2012). By increasing the SRT in biological treatment processes, the slow growing microorganisms can grow and adapt to recalcitrant compounds, which lead to higher biodegradability efficiencies (Clara *et al.* 2004).

Diclofenac was significantly biodegraded when the SRT was at least 8 days (Kreuzinger *et al.* 2004). The removal efficiency of steroid oestrogens in a nitrifying/denitrifying AS WWTP, with phosphorus removal was > 90% (Koh *et al.* 2009). Gobel *et al.* (2007) found that long SRTs in MBRs increased the removal of some of antibiotics (roxithromycin and trimethoprim) from 39% and 33% with a SRT of 16 days to 60% and 87% with a SRT of 60 days respectively. High removal efficiency (> 99%) was achieved for oestrogen in a MBR with a SRT of 60 days (Estrada-Arriaga & Mijaylova 2011).

Overall, it is clear that the biodegradation of pharmaceutical compounds is better when longer SRTs are applied. However, many WWTPs are not designed with sufficiently long SRTs to achieve adequate removal rates (Nghiem *et al.* 2005).

2.8.3 Hydraulic retention time

Studies into the influence of HRT on the removal efficiencies of selected pharmaceutical compounds have reported varied results. Servos *et al.* (2005) found that an increase of the HRT improved the removal of oestrogen, whereas Weiss and Reemtsma (2008) indicated that the HRT does not affect oestrogen removal. Estrada-Arriaga *et al*. (2011) found that an increase in the HRT (to 12 h) during MBR treatments enhanced the biodegradation of oestrogen (close to 100%), as the microorganisms involved had time to adapt. In addition, a longer HRT (24 h) with SRT (10 d) significantly increased the removal efficiency of antibiotics (Kim *et al.* 2005).

2.9 Post-treatment

2.9.1 Activated carbon

Pharmaceutical compounds can be adsorbed by activated carbon (AC) in WWTPs. Several studies have analysed the effectiveness of powdered activated carbon (PAC) and granular activated carbon (GAC) (Gerrity *et al*. 2011; Reungoat *et al.* 2012; Boehler *et al*. 2012; Serrano *et al.* 2011; Altmann *et al*. 2014). Generally, AC is found to have a great potential to remove organic micropollutants from wastewater. Snyder *et al.* (2007) studied the removal of 36 organic compounds by AC, and found that over 90% of the compounds were removed. The removal efficiency of organic micropollutants by AC depends on the properties of the adsorption media and the characteristics of the compounds (Michael *et al*. 2013). According to Ternes *et al.* (2002) the elimination of selected pharmaceuticals (bezafibrate, clofibric acid, carbamazepine, and diclofenac) by AC during a drinking water treatment process was very effective, with the exception of clofibric acid.

Generally, most studies have suggested that higher removal efficiencies (> 90%) of micropollutants can be achieved with AC (Snyder *et al.* 2007; Adams *et al*. 2002 and Le-Minh *et al.* 2010); these including some pharmaceutical compounds that are not readily degraded by microorganisms (Altmann *et al*. 2014). The advantages of AC include its simplicity of application and the possibility of the regeneration, or reuse, of exhausted GAC. However, its efficiency may be reduced by the presence of competing organic compounds in effluents (Altmann *et al*. 2014). This may, in turn, have a negative effect on the

microbial community (Callaway and Aschehoug 2000). It is also a relatively costly form of treatment (Crisafully *et al*. 2008).

2.9.2 Advanced oxidation processes

Advanced oxidation processes (AOPs) are a combination of processes that are mainly aimed at the formation of hydroxyl radicals ($OH^{\bullet}$) (Klavarioti *et al*. 2009). The OH-radicals can oxidize organic compounds including pharmaceutical compounds in wastewater. There have been several studies into the application of AOPs, such as ozone (O_3), hydrogen peroxide (H_2O_2), titanium dioxide (TiO_2) and ultraviolet (UV) techniques, for the treatment of water and wastewater (Ikehata *et al*. 2006; Giri *et al.* 2010; Hübner *et al.* 2013; Sui *et al.* 2014; Sundaram *et al.* 2014). Complete or partial degradation of pharmaceutical compounds in wastewater can be achieved using AOPs, through mineralisation to CO_2 and H_2O, or partial break down to other less harmful compounds. Normally, these technologies can be installed as a tertiary treatment after a biological (secondary) treatment in WWTPs.

In laboratory experiments, Giri *et al*. (2010) examined the removal of 16 pharmaceutical compounds in mixed solutions by seven AOPs (O_3, UV, O_3/UV, O_3/TiO2, UV/TiO_2, UV/H_2O_2, and O_3/UV/TiO2). The ozone-based technique was powerful and removed about 90% of all the compounds, while the combination of O_3/UV was the most appropriate technique for the simultaneous and effective removal of the selected compounds (Giri *et al.* 2010). A review of several studies that applied O_3 to waters contaminated with pharmaceutical compounds indicated that although high degradation efficiency (measured by COD) is achievable, the degree of mineralisation was low and the ecotoxicity of the final

treated water persisted, or even increased (Homem and Santos 2011). Ikehata *et al*. (2006) also assessed the efficiency of AOPs in the removal of several pharmaceuticals from wastewater. They concluded that while some pharmaceuticals were efficiently removed by O_3 (e.g., some antibiotics, carbamazepine, some NSAIDs, and oestrogen 17b-estradiol), other compounds had relatively low resistance (e.g., clofibric acid, diazepam, and ibuprofen), and N4-acetylsulfamethoxazole, a metabolite of sulfamethoxazole, was found to be more resistant to ozonation than the parent compound.

The degradation of pharmaceutical compounds in wastewater that can be achieved with AOPs is dependent on factors such as the oxidant dose, concentration of pharmaceuticals, suspended solids, carbonate, bicarbonate and chlorine ions, pH, and temperature (Ikehata *et al*. 2006; Verlicchi *et al*. 2010b; Homem and Santos 2011). Although, AOPs have the advantage of removing significant concentrations of micropollutants from wastewaters, the mineralisation of certain compounds is still only achieved at low rates, even for long treatment times. In addition, the oxidation process can lead to the increased toxicity of by-products that can also be more resistant than the parent compounds (Andreozzi *et al.* 1999; Dantas *et al*. 2008; Li *et al.* 2008). There are also high equipment and maintenance costs, as well as high energy requirements, for some of the AOPs, which constitute significant disadvantages to the treatment type (Homem and Santos 2011).

2.10 Pharmaceuticals in drinking water

After passing through WWTPs, the residues of pharmaceutical compounds enter receiving waters. Significant concentrations of these compounds in the receiving waters have been reported in several countries (Heberer 2002; Kolpin *et al.* 2002; Choi *et al.* 2008). There may have been instances in which pharmaceutical compounds have contaminated public drinking water through ineffectively treated wastewater being discharged into surface waters that eventually contribute to water supply sources (Benotti *et al.* 2008; Mompelat *et al.* 2009; Jelić *et al*. 2012a). In the summer of 2004, for example, newspapers in the UK reported that Prozac (fluoxetine) was detected in UK drinking water (BBC 2004). Thus, the efficient treatment of surface water before it is used for domestic water supply is also very important to avoid the presence of any pharmaceutical compounds in the drinking water. Table 2.8 shows the range of occurrences of selected pharmaceuticals that have been identified in drinking water (Jelic *et al*. 2012a).

Table 2.8: Concentration ranges of selected pharmaceutical observed in drinking water (Jelic *et al*. 2012a)

Pharmaceutical compound	**Concentration range** (ng/L)
Atenolol	0.84–023
Bezafiberate	1.90–027
Clofibric acid	5.30–270
Gemfibrozil	0.80–070
Diclofenac	1.00–035
Ibuprofen	0.60–008
Ketoprofen	3.00–008
Acetaminophen	45.00–210
Carbamazepine	9.00–258
Phenazone	250.00–400
Propyphenazone	80.00–240

Although, the observed concentrations of the compounds in drinking water (typically in the ng/L range) have not yet reached a serious or a health-threatening level, it is prudent to reduce the levels of these compounds in surface water before any harm is experienced.

2.11 Conclusions

Although there are still many uncertainties about the consumption, occurrence and fate of pharmaceutical compounds emerging in HWW and MWWTPs, a number of conclusions can be drawn from the evidence discussed in this chapter:

- Pharmaceutical compounds have been found in different surface waters all over the world, including rivers, lakes and oceans. Some compounds have then been found in drinking water supplies.
- Hospitals can be significant point sources for some of the pharmaceutical compounds that are released into the water environment.
- The contributions of hospitals to the concentrations of pharmaceutical compounds in municipal WWTPs vary from low (e.g., analgesics) to high (e.g., contrast media and antibiotics).
- Conventional WWTPs can remove some pharmaceutical compounds, while other compounds are persistent and as a result are continuously discharged into surface waters.
- The level of efficiency removal by biological treatments depends on the physico-chemical properties of the compounds, the type of wastewater

treatment technology, the HRT, the SRT and the climatic conditions (e.g. dilution, rainfall, temperature and level of sunlight).

- Although, advanced treatment technologies can be efficient in the removal of most common pharmaceutical compounds, their capital and operational costs are often high.
- Limited information exists on the occurrence and fate of micropollutants in tropical climatic conditions.

In general, considerable uncertainties still remain about the fate of pharmaceutical compounds during common biological treatment processes under different operational conditions (mainly aerobic, anaerobic, SRT, HRT and temperature), which can affect the efficient removal of common pharmaceutical compounds. In order to develop new strategies to improve the treatment of these compounds, more detailed knowledge about the management and influencing factors are required under different conditions.

CHAPTER 3

Materials, methods and analysis

3.1 Selection of pharmaceutical compounds

There are several thousand different licensed pharmaceutical products. The pharmaceutical compounds analysed in the present work were selected from eight different therapeutic classes, as follows: anti-inflammatories and analgesics, antibiotics, contrast media, β-blockers, anaesthetics, antidepressants, cytostatics and lipid regulators (Table 3.1). The compounds were selected from each therapeutic class based on the following criteria:

- For each therapeutic class, the most commonly used pharmaceuticals were selected.
- Potential presence of these compounds in wastewater as hospital drugs.
- Potential effects on aquatic organisms.
- Specific research interests (PILLS project 2012; Helwig *et al.* 2013) and previous measurement in wastewater and surface water (Yu *et al.* 2006; Lin *et al.* 2010).
- The existence of analytical methods able to ensure a sensitive and reliable detection of the compounds during the different experiments.

3.1.1 Analgesics and anti-inflammatories

Paracetamol, naproxen and ibuprofen were selected as appropriate representative compounds of this therapeutic class. Painkillers in general are widely used and can be bought over the counter; paracetamol and ibuprofen

are some of the most commonly used with over 90 and 14 tonnes, respectively, dispensed annually in Scotland (Sniffer 2012). The contribution of hospitals to the overall load at wastewater treatment plants (WWTPs) may be relatively small (up to 30%), due to their general availability to the public (Helwig *et al*. 2013). Ibuprofen and paracetamol have been detected at high concentrations in influents, effluents and receiving waters (Thomas *et al.* 2007; Wu *et al.* 2012). Analgesics, including naproxen and ibuprofen were found in two wild fish species downstream of a WWTP (Brozinski *et al.* 2012) and all three drugs (paracetamol, naproxen and ibuprofen) pose a potential eco-toxicological risk to organisms (PILLS project 2012; Verlicchi *et al.* 2012). Recently, the European Commission added a painkiller (diclofenac) to Directive 2013/39/EU of the European Parliament and of the Council, amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, stating a limit to the annual average value for inland and other surface waters to be 0.1 and 0.01 µg/L respectively (European Commission 2014).

3.1.2 Antibiotics

Five compounds, ciprofloxacin, clarithromycin, erythromycin, sulfamethoxazole and a metabolite of sulfamethoxazole, N-acetyl-sulfamethoxazole (NACS), were selected from the antibiotics therapeutic class (Table 3.1). Antibiotics are a diverse group of chemicals that can be divided into different subgroups such as β-lactams, quinolones, tetracyclines, macrolides and sulphonamides, among others (Kümmerer 2009). They are extensively used by humans for the prevention and treatment of diseases caused by microorganisms.

The contribution of hospitals to the load of antibiotics entering WWTPs vary from low concentrations to up to more than 100%. For example, hospital contributions in the UK have been reported to be about 75% for ciprofloxacin and 36% for clarithromycin; in Luxembourg they were 155% for ciprofloxacin, 53% for sulfamethoxazole and 64% for erythromycin and in Denmark they were 59% for clarithromycin and 82% for erythromycin (Helwig *et al.* 2013). Ciprofloxacin, in particular, is used in European hospitals and is not efficiently removed by WWTPs (Thomas *et al.* 2007).

Antibiotics have adverse effects on aquatic organisms. Their occurrence in wastewaters is correlated to bacterial resistance and a reduction in microbial activity (Niu *et al*. 2013; Herzog *et al.* 2013). In addition to bacteria, algae can be highly sensitive to different antibiotics (Isidori *et al.* 2005; Van der Grinten *et al*. 2010). The genotoxicity of some antibiotic compounds such as ofloxacin and sulfamethoxazole has been demonstrated (Isidori *et al.* 2005). Ciprofloxacin has also been reported to damage the DNA of bacteria in hospital wastewater (Hartmann *et al.* 1999).

The metabolite of sulfamethoxazole, NACS, was selected for this study because high doses of sulfamethoxazole are excreted by humans in the form of NACS (Gobel *et al.* 2005) and it can be converted back to the parent compound during wastewater treatment processes.

3.1.3 Contrast media

Contrast media are mainly dispensed during radiology applications at hospitals. They are excreted by patients (up to 100%) and are highly persistent during

wastewater treatment (Weissbrodt *et al.* 2009). The contributions of hospitals to iopamidol loads to WWTPs have been measured at 100% in the UK (Helwig *et al.* 2013). Therefore, two compounds (diatrizoate and iopamidol) were selected for this work (Table 3.1).

3.1.4 β-blockers

β-blockers, such as atenolol, are used for the treatment of high blood pressure and are prescribed during recovery from heart attacks. Around 90% of ingested atenolol is excreted by patients in the form of the active parent compound (Sniffe 2013). Although this compound is not a typical hospital drug, it has been detected in wastewaters, surface waters and ground waters, indicating that it is not sufficiently removed by WWTPs (Dordio *et al.* 2009). For example, the concentrations of atenolol in effluents from selected hospitals were measured at up to 3.4 µg/L (Gomez *et al.* 2006). An eco-toxicological study showed that most of the β-blockers tested had a specific toxic effect towards green algae (Maurer *et al*. 2007). Atenolol was therefore selected as suitable for this study.

3.1.5 Anaesthetics

Lidocaine was selected for measurement in this study (Table 3.1) because it is widely used as a local anaesthetic. Lidocaine has been detected in wastewaters, surface waters and ground waters (Rúa-Gómez *et al.* 2012). The contribution of hospitals to the lidocaine loads entering WWTPs have been analysed and were found to be 81% and 61% in the UK and Luxembourg, respectively (Helwig *et al.* 2013).

3.1.6 Antidepressants

Carbamazepine is used alone or in combination with other medications to treat mental illnesses, depression, post-traumatic stress disorder, drug and alcohol withdrawal, restless leg syndrome, diabetes insipidus, certain pain syndromes, and a disease in children called chorea (Mohapatra *et al.* 2013). Carbamazepine is not generally administered in hospitals, but it is the most frequently detected pharmaceutical residue in water bodies (Thomas & Langford 2007; Zhang *et al.* 2008). It has also been reported to be ubiquitous in Welsh rivers (Kasprzyk-Hordern *et al.* 2008 a, b). Therefore, carbamazepine is included for further study.

3.1.7 Cytostatics

Cytostatics are anti-cancer drugs that may have carcinogenic, mutagenic, teratogenic and fetotoxic effects (Kümmerer *et al.* 1997). Cytostatics have frequently been detected in water bodies, indicating that they are only partially removed by conventional wastewater treatment plants (Zhang *et al.* 2013). In the UK, the contribution of hospitals to the load of ifosfamide to WWTPs was found to be up to 100% (Helwig *et al.* 2013). Therefore, cyclophosphamide and ifosfamide were selected for study.

3.1.8 Lipid regulators

Bezafibrate is a fibrate drug that is currently used extensively as a lipid regulating agent and has been detected in a variety of different aquatic systems (Yuan *et al.* 2012). The bioaccumulation and biomagnification of bezafibrate

could potentially be very harmful to organisms in the aquatic environment, because of a possible mixture of toxicity, synergistic and additive effects (Li *et al.* 2014). Isidori *et al.* (2007) studied the general toxicity and genotoxicity of fibrates and their photoproducts on organisms (bacteria, rotifers and crustaceans) and found that acute toxicity occurred at concentrations in the order of dozens of mg/L for all the trophic levels tested. However, chronic exposure to these compounds caused an inhibition of population growth in rotifers and crustaceans. Therefore, bezafibrate was selected as suitable for the purposes of this study.

Table 3.1: Pharmaceutical compounds selected for monitoring.

Pharmaceutical class	Compound	PNEC[a] (µg/L)	Brief reasons
Analgesics and anti-inflammatories	Paracetamol	1	• Found in surface water • Bought over the counter • Relatively low PNEC
	Naproxen	2.62	
	Ibuprofen	1.65	
Antidepressant	Carbamazepine	13.8	• Found in surface water • Persistent in WWTPs
Lipid Regulators	Bezafibrate	5.3	• Chronic exposure inhibits growth of some organisms • Relatively low PNEC
Anaesthetics	Lidocaine	82	• High hospital contribution
Antibiotics	Ciprofloxacin	938	• High hospital contribution • High effects on aquatic life • Low PNEC except ciprofloxacin
	Clarithromycin	0.07	
	Erythromycin	0.02	
	Sulfamethoxazole	0.027	
Metabolite of sulfa-Methoxazol	NASCS	n/a	
Contrast media	Diatrizoate	11000	• Up to 100% excretion • Hospital specific substances • Persistent in wastewater treatment
	Iopamidol	380000	
Cytostatics	Cyclophosphamide	11	• Persistent in WWTPs • High hospital contribution
	Ifosfamide	11	
β-blockers	Atenolol	30	• Up to 90% excretion • Found in surface water • Persistent in WWTPs

[a] Predicted No Effect Concentrations (PNEC) from (Verlicchi *et al.* 2012a) except for the contrast media, for which the PNEC are from (PILLS project 2012, cited form http://www.wikipharma.org/api_data.asp)

3.2 Selection locations of wastewater treatment plants

In this research, the fate of pharmaceutical compounds during wastewater treatment is studied in four WWTPs in the UK (Tayside and Fife, Scotland) and two in Saudi Arabia (Riyadh). The choices of these locations were based on different factors, which were as follows:

1. Different management practices at the point source (hospital): while most hospital wastewater (HWWs) are directly released into urban sewerage networks in the UK for co-treatment with municipal wastewater (MWW), HWWs are often pre-treated (on-site treatment) in Saudi Arabia (more details about HWW management are provided in Chapter 4).
2. Different treatment processes: the WWTPs selected represent cross-sections of common types of water treatments in the UK and Saudi Arabia, including activated sludge process (ASP), trickling filter (TF) and tertiary treatment.
3. Different climatic conditions. These can affect key factors in the operational processes, such as the ambient temperature and dilution by from rainwater. The annual average temperatures in the UK are 7–11°C (BBC 2014), while in Saudi Arabia; the average temperature exceeds 26-28°C (Qadir *et al*. 2010; Almazroui *et al*. 2014). Saudi Arabia is an arid region with limited water resources, in contrast to the UK, which has high rainfall. Different climatic conditions (e.g. dilution, rainfall, temperature and levels of sunlight) will have different effects on the biodegradation kinetics during biological treatment, by affecting the microbial activity (Cruikshank & Gilles 2007; Pauwels and Verstraete 2006; Verlicchi *et al.* 2012a; Kasprzyk-Hordern *et al.* 2009b). Therefore,

Saudi Arabia (which has a tropical climate) and UK (which has a temperate climate) were selected for this work as they experience different and contrasting environmental conditions.

3.2.1 United Kingdom

The four municipal wastewater treatment plants (MWWTPs) chosen in the UK (Hatton, Cupar, Guardbridge and Letham) (Figure 3.1) were selected because they encompass a range of service areas (from a large city to a small village) and also use different types of treatment and have different operational characteristics (Table 3.2) that may likely affect the fate of the chosen pollutants in the plants.

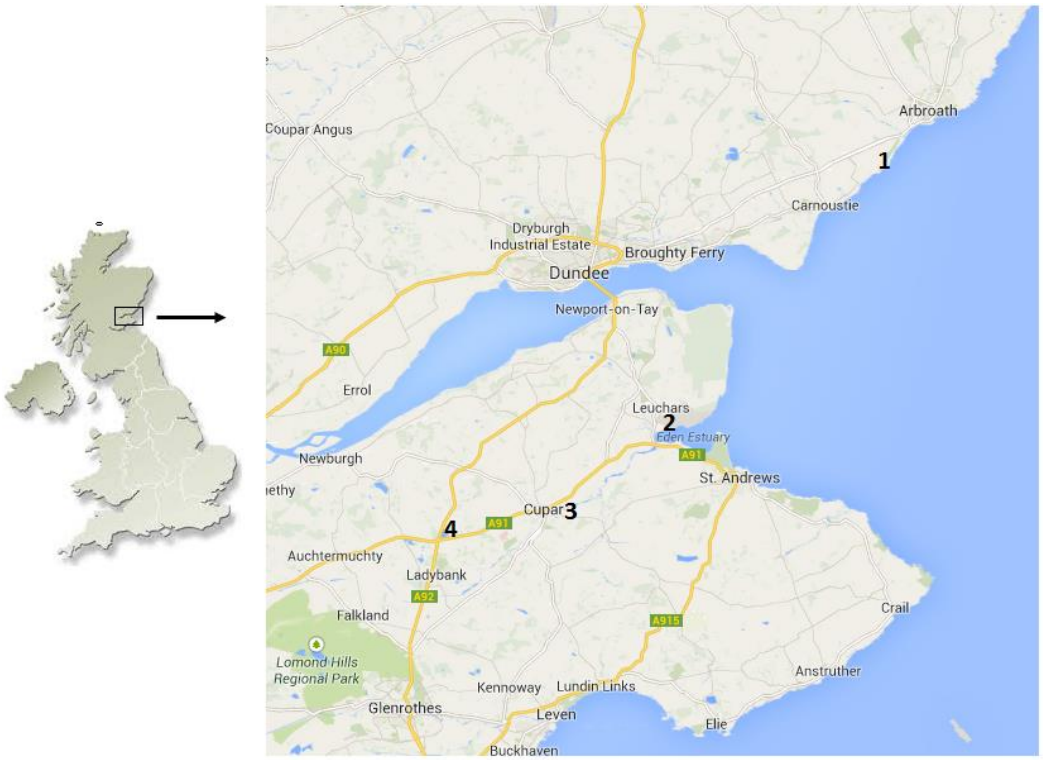

Fig. 3.1: The locations of WWTPs in Scotland, UK (**1** Hatton, **2** Guardbridge, **3** Cupar and **4** Letham).

Table 3.2: Characteristics of selected MWWTPs in Scotland, UK

Parameters	Hatton	Cupar	Guardbridge	Letham
Process technology	Activated sludge	Activated sludge	Activated sludge	Trickling filter
Biological process	Non nitrifying	Nitrifying	Nitrifying	Nitrifying
PE	237,000	16,000	6,000	300
HRT(hours)	4	16	17	n/a
SRT (day)	n/a	9	11	n/a
Ambient Temp (°C)	8±2	8±2	8±2	8±2

PE = Population equivalent; HRT = Hydraulic Retention Time; SRT = Solid Retention Time; n/a = not available

Hatton MWWTP serves a major city (Dundee) and a large town (Arbroath) and processes the wastewater of around 237,000 people. It receives wastewater from multiple different sources (e.g. households, hospitals, shops, industries, veterinary clinics, runoff etc.). According to an email received from Gillian Marnie of NHS Tayside, there are many hospitals in Dundee, including Ninewells Hospital (a major NHS and medical school hospital), King Cross Hospital, Royal Victoria Hospital and BMI Fernbrae Hospital (Table 3.3). Ninewells Hospital is one of the largest teaching hospitals in the UK, with 15 operating theatres, 40 wards, and 5000 employees. The hospital contains about 849 beds and has a full range of healthcare specialties (Healthcare Improvement Scotland 2015). It receives patients from the city of Dundee and the surrounding region. The contribution of these hospitals, like others, to the loads of pharmaceutical compounds at WWTPs may vary based on different administration routes, excretion and the frequency of use of compounds (Helwig *et al.* 2013). The wastewaters from the hospitals that are directly connected to the Hatton plant are co-treated with the MWW.

The Hatton MWWTP employs primary (gravitational settlement) and secondary treatment (aerated sludge) with no nitrification process and secondary clarifier. The treated effluent is discharged into the River Tay. The Hatton plant also treats solid sewage through anaerobic processes.

The Cupar MWWTP is located in Cupar, a town in Fife, and serves a population of around 16000 people. The MWWTP receives raw wastewater from households, shops, two hospitals and veterinary clinics. According to an email received from Norma Aitken of NHS Fife, the hospitals are Adamson and Stratheden, with 186 beds in total (Table 3.3). Both hospitals are community hospitals serving Cupar and surrounding areas. Healthcare services include various specialist units (e.g. physiotherapy, occupational therapy, podiatry, dietetics and speech and language therapy). The Cupar MWWTP employs solids screening, biological treatment with a long aeration time that promotes nitrification, and a secondary clarifier. The final treated effluent is discharged into the River Eden.

Guardbridge MWWTP is located on the River Eden, near the coast, and serves about 6,000 people. Most of the raw wastewater comes from households and shops but hospital wastewater is not included. Treatment at the plant consists of grit removal, biological treatment with a long aeration time that promotes nitrification and a secondary clarifier. The final treated effluent is discharged into the River Eden.

Letham MWWTP serves a small village with a population of less than 300 people. Most of the raw wastewater is generated from households and there is no hospital in the area. The MWWTP employs the following components: solids screening, primary settlement, secondary treatment (trickling filter) and a secondary clarifier. The Letham effluent is discharged into a small lake.

Table 3.3: Hospitals covered by each plant

Plant	Hospital	Bed	Average Inpatient per year	Average consumption water per year
Hatton	Ninewells	849	64164	435232 m^3
	Royal Victoria	72	1244	2261 m^3
	Kingsway Care	59	153	4882 m^3
	Carseview Centre	52	606	7266 m^3
	Fernbrae	15	n/a	n/a
	Bluebell Intermediate care	23	261	n/a
Cupar	Adamson	18	179	1300 m^3
	Stratheden	168	518	9075 m^3
Guardbridge	No hospitals	-	-	-
Letham	No hospitals	-	-	-

3.2.2 Saudi Arabia

The monitoring of wastewater treatment in Saudi Arabia in this study is comprised of two hospital wastewater treatment plants (HWWTPs) in Riyadh, the capital city. HWWTPs are located in the west and south of the city (Figure 3.2). The choice of these locations was based on the following factors; i) they have onsite treatment ii) they provide a whole range of medical services (e.g.

internal medicine, maternity, radiology, cardiology, surgery, kidney disease, dermatology, urology, orthopaedic, paediatric, obstetrics and gynaecology, dentistry, pharmacy and medical laboratory services, among others). The HWWTPs at both hospitals employ ASPs. There are no other HWWPs in Riyadh (or perhaps the entire country) that employ alternative treatment processes. Due to limited access to data, some of the information on operational processes was not available (e.g. hydraulic retention time and solids retention time, among other operational parameters that are commonly measured). The first of the HWWTPs is located in Salman Hospital, while the second one is in Iman Hospital (Figure 3.2). Both HWWTPs are operated by the respective hospital authorities under the direct control of the Saudi Arabian Ministry of Water and Electricity.

Hospital Drugs monitoring: Communication with the pharmacology department (for both Saudi Arabian hospitals) indicated that ibuprofen, naproxen, diatrizoate, iopamidol and fosfamide are not commonly prescribed within either hospital. Therefore, these compounds were not analysed in the influent and effluent samples from these hospitals. In contrast, erythromycin (an antibiotic) was confirmed as being commonly used within the hospitals, so it was selected for analysis.

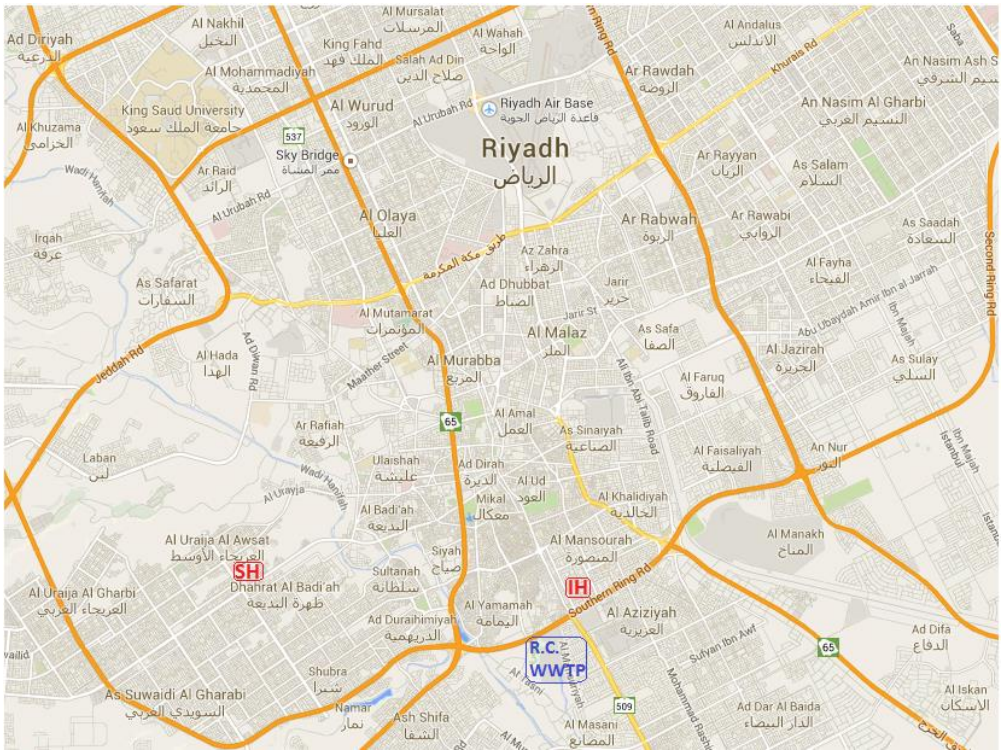

Fig.3.2: The location of on-site HWWTPs selected in Riyadh city, Saudi Arabia. SH: Salman Hospital; IH: Iman Hospital; RCWWTP: Riyadh central wastewater treatment plant (Google Map 2014).

Salman hospital wastewater treatment plant (SHWWTP): Salman Hospital is an important general hospital that employs 3000 people and has 300 beds. At the time of writing, it is being extended to a capacity of 450 beds. Salman hospital covers patients from the west and north of Riyadh and offers a large range of general medical services. In 2013, 8523 inpatients left the hospital. About 328,050 m^3 of water was used in 2013, equivalent to about 896 m^3/day.

The SHWWTP receives wastewater from all of the hospital's departments and systems, including outpatients and inpatients, medical units, restaurants, laundry and the air conditioning systems. The treatment processes the SHWWTP employs consist of screening, aeration tanks (secondary treatment), a secondary clarifier and sand filtration, followed by a chlorination process

(Figure 3.3). The final treated effluent is discharged to the sewerage system that leads to the Riyadh central WWTP.

Imam hospital wastewater treatment plant (IHWWTP): Iman hospital employs 2042 people and contains 215 beds. It also offers a wide range of general medical services, as described above, apart from a kidney disease unit. In 2013, the total water consumption of the hospital was 220,100–230,100 m^3, across all hospital departments, including general services (i.e. restaurants, laundry and air conditioning systems). The IHWWTP processes are made up of screening, primary settlement, aeration tanks, a secondary clarifier, sand filtration and chlorination (Figure 3.3). The final treated effluent is also discharged to the sewerage system for further treatment at the Riyadh central WWTP.

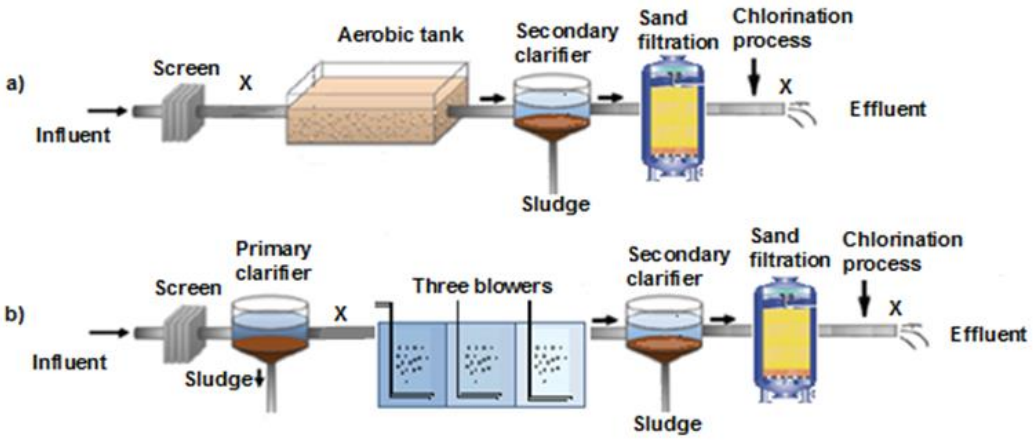

Fig. 3.3: Schematic of the treatment processes employed in Riyadh, (a) Salman Hospital and (b) Iman Hospital wastewater treatment plants (X = sampling point).

3.3 Sample collection

The removal of pharmaceutical compounds from WWTPs occurs either by biodegradation and/or adsorption onto sludge. Most of the removal of pharmaceutical compounds occurs as a result of degradation (Ternes *et al.* 2004; Radjenović *et al.* 2009). Removal by adsorption onto sludge is generally quite low (<20%) except for some antibiotics (Verlicchi *et al.* 2012a,b). In addition, pharmaceutical compounds have little tendency to adsorb onto sludge at neutral pH (Uraes and Kikuta 2005). Therefore, although a bigger picture would be gained by analysing the sludge and liquid samples of each WWTP, only aqueous samples were collected and analysed in this research due to financial limitations.

3.3.1 United Kingdom

Samples were collected from two points (before and after secondary treatment) as marked by (X) in Figure 3.4. Sampling was carried out in winter (3 October–9 November 2012) and also in summer (3–28 June 2013). A minimum of two samples were taken weekly for four weeks in winter (with an average air temperature of 6.5°C) and also for four weeks in summer (with an average air temperature of 13.2°C) from each site over a 5 hour period (09.00 h–14:00 h). All the samples were collected in 500 mL sterile plastic bottles (UK Water, UK) and immediately transferred to the laboratory for the analysis of conventional parameters. 150 ml of each sample was frozen at –20^{o}C for the analysis of the pharmaceutical compounds. Therefore, across all time points 1200 mL of sample was obtained from each sampling point at each site (for example, a

total of 600 mL influent collected from Cupar plant in winter was mixed with the corresponding 600 mL collected in summer time) for every plant. Due to the expense of analysing pharmaceutical compounds, the winter and summer samples taken at each sampling point were mixed together to obtain average values over both seasons.

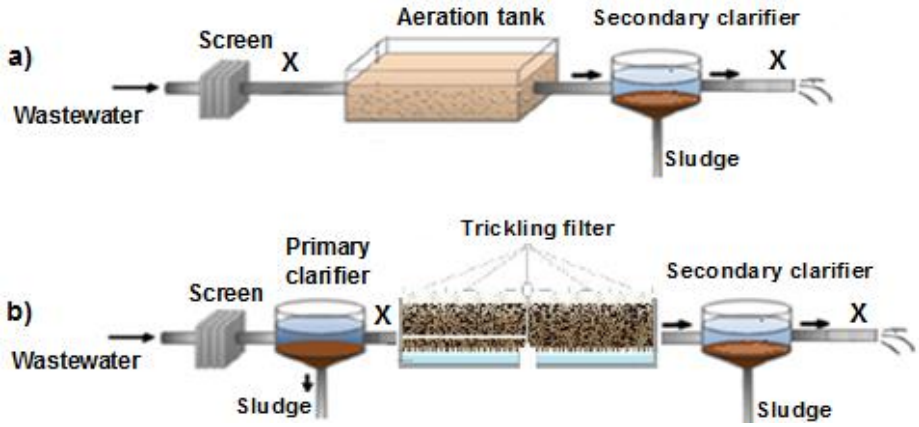

Fig. 3.4: Schematic of the treatment processes employed in UK, (a) activated sludge and (b) trickling filter (X = sampling point).

3.3.2 Saudi Arabia

Samples were collected from two points, before the secondary treatment and after the tertiary treatment process as marked by (X) in Figure 3.3. Sampling was carried out twice weekly for four weeks in April 2014. Samples were collected in the middle of the day (11:00-14:00) when the air temperature was at its highest (30-35 °C). This is in anticipation that the temperature of the surface water would have been warmest at this time of day. The samples were collected in 1 L sterile plastic bottles (Saudi Water, Saudi Arabia) and transferred in a cool box to the laboratory at the School of Science, King Saud University (KSU) for the analysis of conventional parameters. 400 ml of each

sample were frozen at –20^{o}C for the analysis of the pharmaceutical compounds.

All the samples collected from each respective sampling point were mixed together and three aliquots of 1000 mL were taken for analysis of the pharmaceutical compounds.

3.3.3 A note on sampling

Excreted drug residues in sewers can be subject to high short-term fluctuations, due to toilet flushes and diurnal variations (Ort *et al.* 2010). The influence of the sampling methodology on data accuracy depends on the number of pollutant peaks and the sampling frequency; sampling intervals of five minutes or shorter may be required to properly account for temporal variations in the pharmaceutical compounds present in influents and to ensure that no toilet flushes are missing (Ort *et al.* 2010). In addition, Gardener *et al.* (2013) indicated that, a minimum of two samples are necessary from each site over a 12-hour period in order to assess within-day variability. However, in this study, these potential short term variations in pharmaceutical compound loading were not put into account due to restrictions with regard to time and the availability of sampling facilities. Furthermore, mixing of the samples as explained in sections 3.3.1 and 3.3.2 was carried out in an attempt to cost effectively reduce the effects of the fluctuations. Therefore, collected sampling uncertainty may add some variation to the concentrations of pharmaceuticals in samples.

3.4 Analysis of conventional parameters

The chemical oxygen demand (COD), total organic carbon (TOC), nitrate (NO_3) and ammonium (NH_4) were analysed according to the manufacturer's instructions for the Spectrophotometer, model DR 5000 (Hach Lange, Germany). Biochemical oxygen demand (BOD_5) was analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998).

3.5 Analysis of pharmaceutical compounds

The pharmaceutical compounds were analysed as described by Helwig *et al*. (2013) and illustrated in Figure 3.5. This method was developed by the School of Engineering and Built Environment, Glasgow Caledonian University (GCU) and was chosen to ensure the detection of the compounds of interest.

3.5.1 Sample preparation

In the UK, the samples were prepared for LC-MS/MS analysis by filtration, extraction, elution and drying down. The wastewater samples (1000 mL) were filtered through 100 µm, 1.6 µm and 0.7 µm glass microfiber filters (Whatman, UK) and then a 0.45 µm cellulose nitrate membrane sterile filter (Whatman, UK), to remove any suspended particulate matter. Each filtered sample was adjusted to pH 2.0 (± 0.1) through the addition of 0.5 M hydrochloric acid. Solid phase extraction (SPE) cartridges, Strata-X, 1 g/20 mL, 33 polymeric reserved phase (Phenomenex, UK) were pre-conditioned with methanol (2 mL) and distilled water (2 mL). Then, the samples were loaded at a flow rate of 10

mL/min, using an SPE vacuum manifold with 12 holes (Macherey-Nagel, Germany). The cartridges were dried under vacuum and washed 3 × 2 mL with water, before being eluted with 4 × 2 mL CH_3CN/MeOH containing 0.1% formic acid. The samples were then dried down under nitrogen. The dried-down samples were re-constituted in CH_3CN/H_2O (30/70). The samples were then diluted further (1:8) by removing 100 ul aliquot and adding 700 ul CH_3CN/H_2O. Deuterated internal standards were added to afford a concentration of 5 ug/L prior to liquid chromatography/tandem mass spectrometry analysis.

Triplicate samples (1000 mL) from the HWWTPs in Saudi Arabia were partially processed (filtration and extraction) at the School of Science, King Saud University (KSU). The same processes and materials (filters and cartridges) as those described above were applied, with one difference: the samples were loaded at a flow rate of 10 mL/min using an SPE vacuum manifold with 24 (instead of 12) holes (Waters USA). At this point, the cartridges were dried under vacuum for a few minutes and then frozen at −20°C. Then, all the cartridges were transported back to the UK (Abertay University) in a cool box (journey duration about 9.5 hours). All of the remaining steps (elution, drying down, reconstitution and the incorporation of standards) were carried out as above.

3.5.2 HPLC-MS analysis

The extracts for all samples were analysed at Glasgow Caledonian University. Two different types of equipment were used. The UK samples were analysed by LC-MS/MS in 2013. The equipment used for this analysis became faulty and

therefore the Saudi Arabian samples were analysed in 2014 using new equipment, as described below:

- *UK samples*

Chromatographic separation of the analytes was performed by LC-MS/MS (Agilent 1100 series LC system, autosampler) using an Atlantis dC18 3 µm, 2.1 × 150 mm column (Waters, UK). A binary gradient with a flow rate of 0.2 mL/min was used; the mobile phase A used was acetonitrile and the mobile phase B was ammonium formate (10mM)/formaic acid/water, adjusted to pH 3.5. The gradient was run from 1% to 99% organic over 45 mins. The column oven was maintained at 30 ± 2°C. The sample injection volume was 10 µL and the auto-sampler was operated at room temperature. The detector was an Esquire 3000 plus Ion Trap mass spectrometer (Bruker Daltonics UK).

- *Saudi Arabia samples*

Chromatographic separation of analytes was also performed by LC-MS/MS (Thermo Scientific Q Exactive Quadrupole-Orbitrap Mass Spectrometer) using an Xselect HSS T3. 2.1 × 150 mm column (Waters, UK). The same flow rate, mobile phases, and gradient were used, but this was carried out over 33 mins (instead of 45 mins).

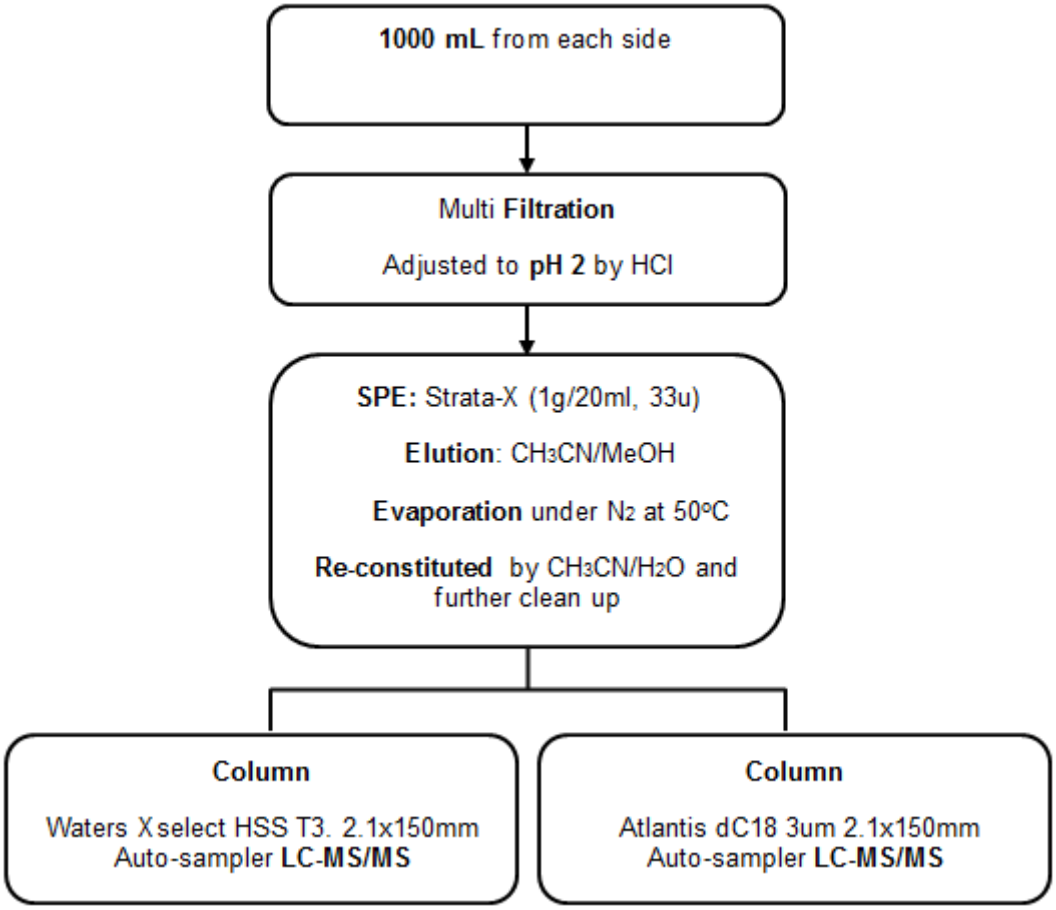

Fig.3.5: Schematic of the analytical methods performed to determine the concentrations of pharmaceutical compounds in the WWTP samples.

3.6 Biodegradation experiments

Short-term batch biodegradability tests were conducted for selected target pharmaceutical compounds in order to evaluate their individual fate under biological aerobic and anaerobic conditions. These biological conditions occur in wastewater treatment systems, the wastewater transport in sewers, storage and in the key treatment units of the wastewater treatment plants selected for this study (as indicated in sections 3.2.1 and 3.2.2). The concentrations of pharmaceutical compounds in wastewater are normally between µg/L and ng/L. In order to check the representativeness of studies at concentrations that are

more readily tested and analysed, a markedly higher concentration of 1 mg/L was used for pollutants in experiments.

3.6.1 Chemical compounds

The pharmaceutical compounds were obtained from Sigma-Aldrich (Sigma-Aldrich®, UK): paracetamol (CAS-Number 103-90-2), ibuprofen (CAS-N. 15687-27-1), naproxen (CAS-N. 22204-4-531) and sulfamethoxazole (CAS-N. 723-46-6). Due to the pharmaceutical compounds have sparing solubility in water, methanol was used to dissolve each of the compounds and a stock solution was prepared that contained all of the pharmaceutical compounds.

3.6.2 Synthetic wastewater

Synthetic wastewater was used for the biodegradation experiments (Table 3.4). The composition of the synthetic wastewater used in this study is based on a synthetic feed with characteristics from Shanmugam and Akunna (2008), with minor modifications.

3.6.3 Seed biomass

For the aerobic biodegradability experiment, seed biomass was collected from the secondary effluent of the Guardbridge MWWTP (an activated sludge process plant) in 500 mL sterile plastic bottles (UK Water, UK) and transported directly to the laboratory. The seed effluent was stored at 4°C for less than 24 hours before being used. The seed effluent was collected from the Guardbridge

MWWTP because the plant applies ASP that has a longer SRT and HRT (Table 3.2).

For the anaerobic biodegradability experiment, because Hatton MWWTP is the only plant that also has an anaerobic sludge digestion process for sludge treatment, anaerobically digested sludge seed (1000 mL) was collected from there and transported to the laboratory, where it was stored at 37°C. The characteristics of the sludge at the time of inoculation in the experiments were: pH = 7.70, total solids (TS) = 25.21 g/L, volatile solids (VS) = 14.29 g/L and volatile fatty acids (VFA) = 259 mg/L.

Table 3.4: Composition of synthetic wastewater.

Name	**Compound**	**Concentration mg/L**
Ammonium Bicarbonate	NH_4HCO_3	200
Potassium Phosphate	KH_2PO_4	80
Glucose	$C_6H_{12}O_6$	10
Magnesium Sulphate	$MgSO_4$	1
Iron	$FeCl_3$	1
Calcium chloride	$CaCl_2$	1
Potassium chloride	KCL	1
Cobalt chloride	$CoCL_2$	0.2
Nickel chloride	$NiCL_2$	0.2
BOD_5		11-15
TS		190-220

3.6.4 Aerobic biodegradability batch tests

Two aerobic batch tests were conducted in 275 mL bottles. Each bottle contained synthetic wastewater (10 mL), seed effluent (10 mL) and the drug

stock solution (1 mg/L), and was made up to 275 mL using fully aerated dilution water. The bottles were kept in an incubator at 20 ± 1°C. Samples (200 mL) were taken at different retention times in order to detect the concentration of the selected pharmaceutical compounds remaining. The aerobic batch tests were performed at 20±1oC in this study, which is conventional standard temperature usually used in wastewater organic aerobic biodegradability tests (Dytczak et al. 2006; Myers & Wilde 2003; SWITCH 2008).

3.6.5 Anaerobic biodegradability batch tests

Duplicate batches and blank cultures were prepared for pharmaceutical compounds using different retention periods. Sludge (1000 mL) from the Hatton anaerobic sludge process was added to 2000 mL of synthetic wastewater and the final mixture volume was made up to 3500 mL with distilled water. The pharmaceutical compound stock solution (1 mL/L) was added into the mixture after a blank sample was taken. Then, 200 mL samples were withdrawn and put into 500 mL bottles. These bottles were purged with nitrogen gas for 2 minutes (in order to displace the oxygen), then each was sealed with a rubber septum and an aluminium cap. The cultures were then incubated at 37°C (commonly used optimum mesophilic temperature for anaerobic wastewater treatment processes (Musson *et al.* 2010; Carballa *et al*. 2007; Angelidaki & Sanders 2004). Each bottle was manually shaken a few times every 24 hours. Samples (200 mL) were taken to determine the pH and the concentration of the pharmaceutical compounds.

3.6.6 Sample analysis

The fates of the pharmaceutical compounds in the batches were analysed using the same method that was employed to monitor the compounds in the UK MWWTPs, as reported above (section 3.5.1 and 3.5.2) and is illustrated in Figure 3.6.

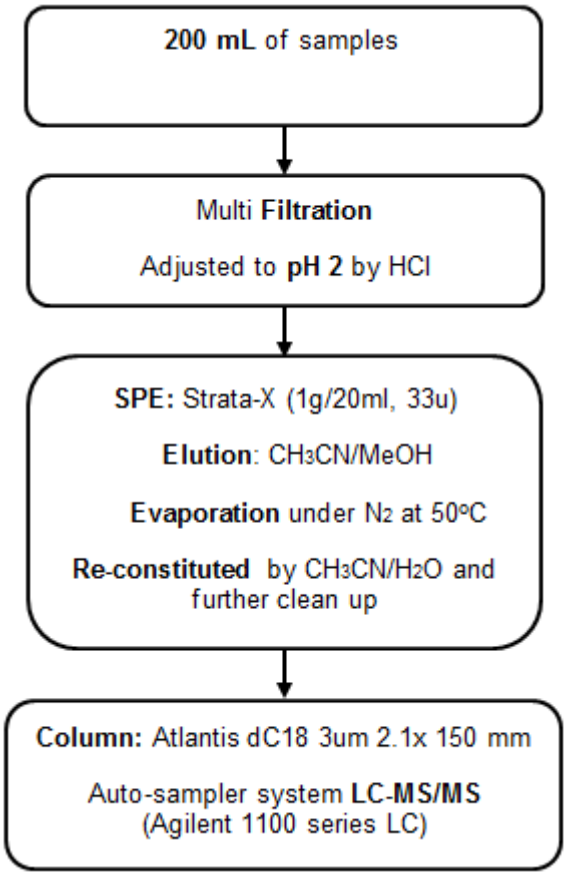

Fig. 3.6: The analytical method applied to determine the biodegradation of selected pharmaceuticals in batch experiments.

CHAPTER 4

Hospital Wastewater Management in the UK and Saudi Arabia

4.1 Introduction:

Wastewater treatment plants, especially those receiving hospital wastewater, are important point sources of entry of pharmaceuticals compounds into the aquatic environment. In many countries, HWW is connected with urban sewage and co-treated in MWWTP. However, in some countries on-site hospital wastewater can receive pre-treatment and legislation in the field of water, wastewater and the environment may be different from country to country.

Until recently, little has been reported on the performance of different wastewater treatment processes, in various parts of the world, in the removal of pharmaceutical compounds. In order to contribute to the limited data on the subject, two different locations (UK and Saudi Arabia) have been selected as part of this study to assess the performance of current management and treatment practices on the treatment of selected pharmaceutical compounds often found in some municipal and hospital wastewaters. This section reviews current common management and treatment strategies of hospital wastewater including the collection, treatment and disposal.

4.2 Current hospitals wastewater management in the UK

Several thousand pharmaceutical compounds are used for a range of purposes worldwide. In the UK, there are about 3000 active substances that are licensed for use (Barcelo 2005). In hospitals, a wider variety of chemicals, in addition to

pharmaceuticals are used for medical purposes. These compounds are used in very large volumes and their consumption is rising year by year (Chapter 2). Pharmaceutical compounds in receiving waters have been linked to the disruption of the sexual activities of fish in UK waters (Van Aerle *et al.* 2001; Cheshenko *et al.* 2008). Effective and comprehensive hospital wastewater management is thus important in reducing environmental risks related to pharmaceutical compounds.

To control the release of these compounds in the environment in the UK, Water UK recently issued National Guidance for Health Care Wastewater Discharges (NGHCAD) (Water UK 2011). This guidance clarifies requirements for preventing environmental harm from hazardous pollutants.

4.2.1 Hospital wastewater collection

In the UK, most hospital sewers are directly connected to the urban sewer and both hospital and municipal wastewater are co-treated in municipal wastewater treatment plants (MWWTPs). Sewer networks cover about 96% of the UK population, and the remaining 4% represents small communities and individual properties in rural areas not covered by sewer networks (Defra 2012). There is more than 624,200 km of sewer networks in the UK that collect wastewater from homes, industries, rainwater run-off from roads and from other sources, including healthcare services. There are three main methods of wastewater collection in the UK (Defra 2012):

- Surface-water drainage (rainwater run-off from roads and urban areas are collected and discharged into local receiving water).

- Combined sewerage (rainwater run-off is collected with wastewater from domestic, industrial, commercial and other premises, including hospital wastewater).
- Foul drainage (wastewater only).

Both surface water and foul drainage may eventually connect to combined sewerage. The combined sewerage system is not uncommon in the UK as well as other countries in Europe (Defra 2012).

According to the NGHCWD guidelines, there are three categories that healthcare products will be classified into with regards to discharge into the UK sewerage system:

- Discharge into the sewer is prohibited (e.g. excess liquid anesthetics in syringes, pharmaceuticals classified as cytotoxic/cytostatic and radioactive materials are not allowed to be discharged to sewers) and should be appropriately disposed.
- Approval is required before discharge to sewer (e.g. preservatives, fixatives and test pharmaceutical products or substances used in their manufacturing); in this case, the hazardous properties of the product ingredients are not fully known. Therefore, hospitals should ask the local sewerage operator for decisions regarding discharges.
- No prohibition on discharge to sewer (e.g. small quantities of reagents, non-pharmaceutically active products and domestic services).

In addition, NGHCWD further stipulates a strong recommendation for hospitals to prepare emergency plans for any spillage status, loss of water supply and drainage plans for any materials that are prohibited.

4.2.2 Hospital wastewater treatment

There are about 9000 WWTPs in the UK, involving some or all of the following four main stages (Defra 2012):

- Preliminary treatment
- Primary treatment
- Secondary treatment (biological treatment)
- Tertiary treatment: using advance treatment processes to reduce different pollutants.

In order to determine the required stages for any WWTP, the population equivalent (PE) and type of receiving water are the most important factors to consider according to the European Commission Urban Wastewater Treatment Directive (EC-UWWTD) as shown in Table 4.1 (EUWWTD 2006). As reported above, hospital wastewater is normally discharged into the public sewer system and both hospital and municipal wastewater are co-treated in MWWTPs

Table 4.1: Treatment stages for WWTPs in the UK (EUWWTD 2006)

Population Equivalent	By end of	Receiving water	Specified Treatment
> 150,000	2000	Less sensitive coastal waters	Primary
	2000	Normal*	Secondary
	1998	More sensitive	Secondary and possible N&P reduction **
15,000-150,000	2000	Less sensitive coastal waters	Primary
	2000	Normal	Secondary
	1998	More sensitive	Secondary and possible N&P reduction
10,000-15,000	2005	Less sensitive coastal waters	Primary
	2005	Normal	Secondary
	1998	More sensitive	Secondary and possible N&P reduction
2,000 10,000	2005	Normal costal	Appropriate***
	2005	Less sensitive estuaries	Primary
	2005	Other estuaries & freshwater	Secondary
< 2,000	2005	All receiving water	Appropriate

***Normal:** receiving water is categorized as neither less nor more sensitive, and there are no detrimental effects on environment. ****N&P:** reducing nitrogen and phosphates. *****Appropriate:** any treatment allowing receiving water to meet quality objectives required.

4.2.3 Disposal of treated wastewater

All wastewater treatment plants must get a permit from local Environmental Protection Agencies (e.g. Environment Agency in England and Wales, Scottish Environment Protection Agency in Scotland, Northern Ireland Environment in North Ireland) in order to discharge effluent into the environment (Water UK 2011). Generally, the treatment required for discharging final treated effluent to

the environment depends on the receiving waters and the population equivalent as shown in Table 4.1.

4.2.4 Sewage sludge disposal

Approximately 80% of sewage sludge is used as a soil enhancer and fertilizer on agricultural land. The quantity of sewage sludge used for this purpose is generally increasing in the UK as shown in Table 4.2 (Defra 2012).

Table 4.2: Summary of Sewage sludge re-uses and disposal routes in the UK (Defra 2012)

Reuse	Sludge Reused (Tons dry solids)		Sludge Disposed (Tons dry solids)			Total
	Soil & Agriculture	Other	Landfill	Incineration	Other	
1992	440137	32100	129748	89800	24300	997673
2008	1241639	90845	10882	1858902	1523	1530779
2010	1118159	23385	8787	259642	2863	1412836

The disadvantage of sludge use in agriculture is that, some pharmaceutical compounds are adsorbed onto sludge during wastewater treatment and sometimes later released into the environment through the transportation of sludge particles and their application in agriculture (Clarke and Smith 2011). Chemicals discharged from industrial, domestic and urban sources into the urban wastewater collection system, are sometimes transferred to the residual sewage sludge during wastewater treatment. As stated previously in page 25, the anaerobic digestion process is used to treat sludge in the UK. Studies have shown that, norfloxacin, ofloxacin, ciprofloxacin, doxycycline, clotrimazole, ketoconazole and econazole compounds were detected in sludge samples at

less than 1 mg/kg dry weight (Lindberg *et al*. 2005; Golet *et al.* 2002). Clarke and Smith (2011) estimated the concentrations of pharmaceutical compounds in agricultural soils amended with biosolids to be less than1 mg/kg dry weight, which is significantly below the soil predicted no effect environmental concentration (PNEC) values.

4.3 Wastewater management in Saudi Arabia

Saudi Arabia is a hot and dry country located in an arid region where water resources are limited and the demand for water increases continuously. Ambient temperatures in Saudi Arabia during summer can range between 30^{o}C and 55^{o}C (Almazroui *et al*. 2012). In Saudi Arabia, it is not only households that consume clean water but also industry and agriculture. Reusing wastewater for agriculture and industry is a solution for the problem of limited water resources. However, wastewater needs to be treated at a high level before use. In Saudi Arabia, hospital wastewater is often pre-treated onsite in some cities without co-treatment with municipal wastewater as reported in UK.

This section provides an overview of the geography and climate of Saudi Arabia and an insight into its approach to water and wastewater treatment and management. Also, it reviews hospital wastewater management including collection, treatment and disposal strategies.

4.3.1 Description of Saudi Arabia

Saudi Arabia is located in South West Asia, it occupies the majority of the Arabian Peninsula, and is at the crossroads between Asia and Africa, with a

land area of 2,149,690 km^2 (Figure 4.1). Saudi Arabia is the largest country on the Arabian Peninsula and extends from the Red Sea in the west to the Arabian Gulf in the east. The population was estimated in 2012 to be about 29,195,895, and the annual population growth rate is about 1.5% (MOH 2012).

Riyadh is the capital and the largest city of Saudi Arabia. It is located in the centre of the country and is one of the 13 provinces of Saudi Arabia. Riyadh is a relatively warm city and classified as having a hot desert climate, with an average annual temperature of above 26ºC (Figure 4.2). During the summer months, the temperature is extremely hot, approaching 50°C occasionally. The annual rainfall in Riyadh is very low, especially in summer, with an average 8 mm annually, while the average total rainfall in Saudi Arabia is no more than 60 mm. The population of Riyadh is estimated at 7,309,966 inhabitants, occupying an area of 1,554 km^2. Riyadh is governed by the Riyadh Principality, which appoints the executive committee that runs the everyday management of the city. In Riyadh, there are about 60 government hospitals, 435 government general practitioners, 47 private hospitals and 720 private GPs (excluding dental clinics) (MOH 2012). The Ministry of Health (MOH) manages most of these hospitals. Some hospitals have on-site wastewater treatment plants while others are directly connected to municipal wastewater treatment plants.

Currently, only about 37% of Saudi Arabia is covered by a sewerage system and the length of the network sewerage system in 2012 was about 25,791 km (MOWE, 2012). However, the country has set the target of achieving 100% coverage of sewerage establishment across the whole country by 2025 (KAUST 2012). It is expected that, with the forecasted increasing population growth in

the country, wastewater generation is projected to increase, thereby necessitating the need for greater sewerage coverage.

Fig. 4.1: Saudi Arabia map. Source (Gause 2011)

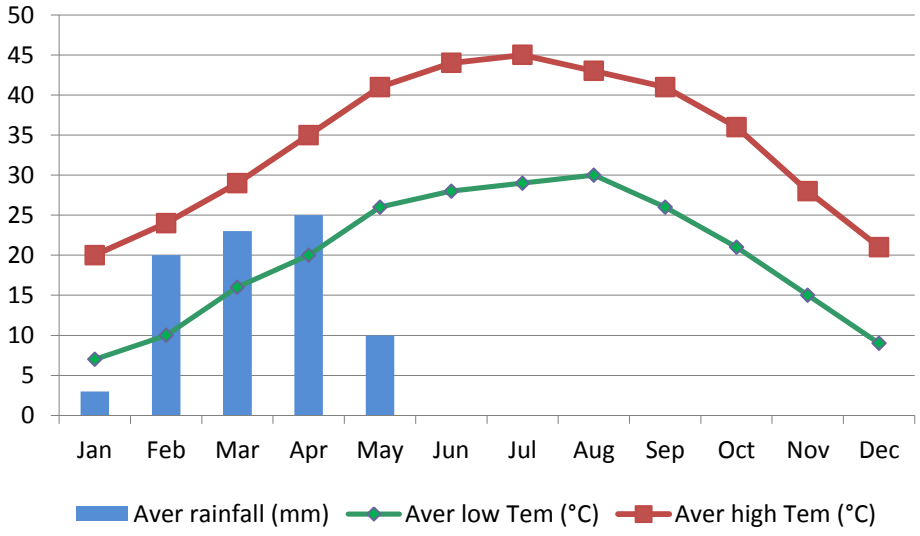

Fig. 4.2: Average temperature in Riyadh city, Saudi Arabia (BBC 2014)

4.3.2 Conventional Wastewater Treatment in Saudi Arabia

The total amount of drinking water supplied by the Ministry of Water & Electricity (MOWE) to all the cities of Saudi Arabia was estimated at 2,527 million m^3 in 2012, and the total flow of municipal wastewater collected in sewer networks and passed through wastewater treatment plants was about 1,257 million m^3 in the same year, as shown in Table 4.3 (MOWE, 2012). This implies that the amount of treated municipal wastewater is equivalent to 50% of the total amount of municipal water delivered. Also, it is worth noting that not all urban water requirements are provided by MOWE. In many urban and rural areas, water supplies are provided from private sources, such as wells, other than those operated by MOWE. Another factor to be considered is the fact that part of the wastewater collected from urban areas is collected as a result of extensive infiltration to the sewer systems due to the rising water table (perched water) (Al-Rehaili 1997).

Table 4.3: Total water and wastewater in 2012 in Saudi Arabia and Riyadh city (MOWE 2012)

	Riyadh city	**Total in Saudi Arabia**
Total water consumption	758 million m^3	2527 million m^3
Average human consumption daily	285 L/day	238 L/day
Total wastewater production	n/a	2021 million m^3
Total wastewater treated	349 million m^3	1257 million m^3

The municipal wastewater disposal in city areas is accomplished by a mixture of sewers and septic tanks. In small towns and rural areas the disposal is limited to septic tanks only (Abu-Rizaiza 1999). By 2011, 70 wastewater treatment

plants (WWTP) had been built in the cities of Saudi Arabia to treat the collected wastewater from the network for safe disposal or reuse (MOWE 2014). The level of wastewater treatment required (secondary and tertiary treatment) is dependent on the intended end use. Generally, all wastewater is required to be treated to at least secondary levels prior to reuse, producing varying degrees of purity.

Secondary treatment usually encompasses the activated sludge process and clarifier in a conventional wastewater treatment plant. Tertiary treatments like sand filtration and disinfection by chlorination, or other processes, are required when water is reused for irrigation. The chlorination process has become necessary in most conventional WWTPs (secondary or tertiary treatment). This is due to the fact that the potential for human contact has increased and chlorination can assist the removal of pathogens. Some effluent from WWTPs is used for restricted and unrestricted irrigation, public parks, medians of roads and highways, etc. A wide range of pharmaceutical compounds has commonly been detected in wastewater effluents in the US and Europe, and they have also been detected in effluents from conventional WWTPs in Saudi Arabia (Shraim *et al.* 2012 and Alidina *et al.* (2014). A case study carried out on four municipal WWTPs in western Saudi Arabia found a high concentration of pharmaceutical compounds (up to 16 ug/L) observed in the effluents of non-nitrifying biological treatment plants (Alidina *et al*. 2014). However, no study has investigated the occurrence and efficient removal of pharmaceuticals compounds in hospital wastewater within Saudi Arabia.

4.3.3 Water reuse in Saudi Arabia

Middle Eastern and North African (MENA) countries, including Saudi Arabia, are located within arid and semi-arid regions, and contain only 1% of the world's freshwater, making them the driest countries in the world (Qadir *et al*. 2010). The MENA countries suffer from harsh water shortages and have very few sources of natural freshwater. In Saudi Arabia, almost 83% of the fresh water is used in farming (Abderrahman 2001). Consequently, groundwater has been over-exploited during the last 30 years. In order to meet the growing demand for water in the agriculture sector, the use of reclaimed wastewater for agricultural irrigation in Saudi Arabia has increased from 123 million m^3/year in 2006 to 225 million m^3/year in 2011 (MOWE 2012). Currently, Saudi Arabia intends to put in place new policy initiatives that will motivate people to put their trust in reclaimed wastewater, thus, guaranteeing a substantial percentage of the nation's future water supply (Jacobsen *et al*. 2012).

The Ministry of Water and Electricity (MOWE) together with the Ministry of Municipalities and Rural Affairs (MMRA) have relaxed their rules regarding wastewater reclamation in order to expedite these reforms (Al-Jasser 2011, cited from Aljassim 2013). Nevertheless, wastewater reclamation in Saudi Arabia is a relatively recent development and a large amount of the reclaimed water is released into the sea and dry rivers beds. The primary use of reclaimed wastewater was initially in landscaping. Later, reclaimed wastewater began to be used in irrigation. On a pilot-scale level, the recycled water is used to replenish the groundwater and safeguard the aquifer reserves, taking into account the possible harmful effects it may have on the health of the public and the quality of the groundwater. Reclaimed wastewater has also been used for

irrigating trees (palm) and fodder crops. Table 4.4 shows the Saudi Arabian regulations for raw wastewater entering treatment networks and the reclaimed wastewater that is used for irrigation purposes (MOWE 2014).

At present, treated wastewater is only being used for irrigation in specific development programs for crops such as wheat, fodder, orchards and palm trees. Bearing in mind that these irrigated crops create products that commonly serve as food for livestock as well as humans, it is crucial that the reused water is thoroughly treated before use.

Table 4.4: Saudi Arabian regulation of wastewater distribution entering treatment networks and reclaimed wastewater for irrigation purposes (MOWE 2014).

pollutants	Unit	Raw wastewater entering plant[a]	Reclaimed wastewater	
			Restricted Irrigation[b]	Unrestricted Irrigation[c]
Physical parameters				
TSS	mg/L	600	40 [d]	10 [d]
TDS	mg/L	-	2500	2500
pH	-	6-9	6-8.4	6-8.4
Turbidity	NTU	-	5	5
Chemical parameters				
BOD_5	mg/L	500	40[d]	10[d]
COD	mg/L	1000	-	-
TOC	mg/L	400	-	-
TDS	mg/L	-	2500	2500
NH_4-N	mg/L	80	5	5
NO_3-N	mg/L	-	10	10
Biological parameters				
Faecal Coliform	CFU/100 ml	-	1000[d]	2.2[d]

[a] Raw wastewater: Indicative raw wastewater related to large communities and companies that are not covered by sewage networks.
[b] Restricted Irrigation: Can irrigate all types of crops except vegetables, tuber crops, and any fruit can touch treated water.
[c] Unrestricted Irrigation: Can irrigate all types of crops without exception.
[d] Monthly average should not be more than limit in table

4.4. Current hospital wastewater management situation in Riyadh city: a review

4.4.1 Hospital wastewater collection

There are three options that are used for hospital wastewater collection and treatment operating in Riyadh, as shown in Figure 4.3 (a-c):

- On-site hospital wastewater treatment plant: The most common type of treatment, where wastewater generated from the hospital is treated within the hospital facility prior to discharge into the municipal wastewater sewer. Although this scenario is likely to be costly relative to other possible options, it is considered to be the best option in terms of hazard reduction from point source (hospital) as a consequence of the two stage treatment level and maximal safety (Pauwels and Verstraete 2006).
- Municipal treatment: Alternatively, rather than onsite treatment, hospital wastewater can be directly connected to urban sewerage and co-treated in the municipal WWTP. This scenario is similar to hospital wastewater management in the UK.
- Onsite collection: The hospital wastewater is collected into ground tanks located at the hospital facility. The raw wastewater is collected periodically for treatment at the central municipal WWTP. The third scenario is applied when the hospital is not covered by a sewerage system.

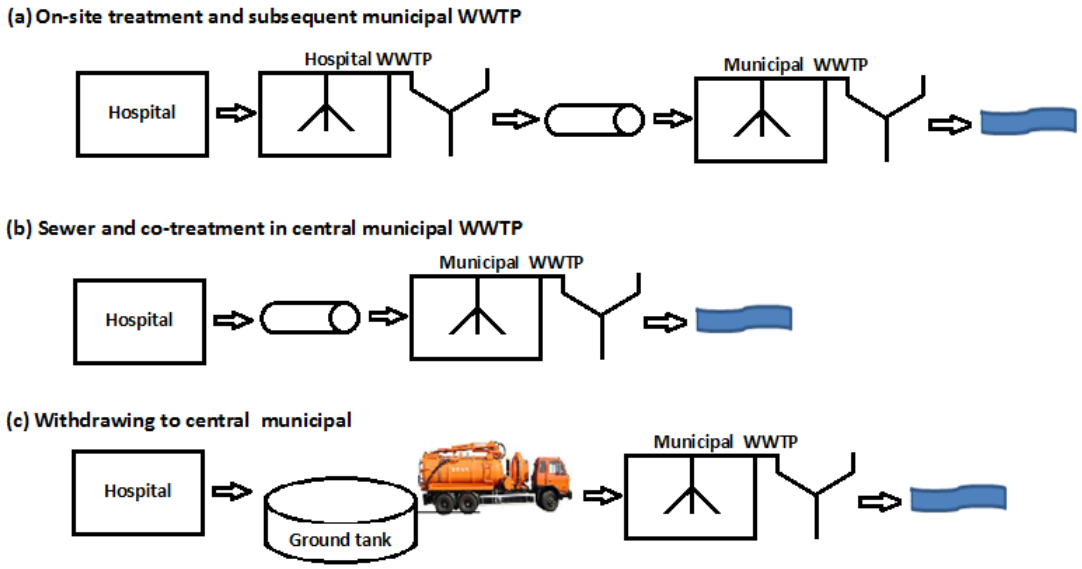

Fig. 4.3 (a-c): Scenarios of hospital wastewater treatment, management and disposal in Riyadh, Saudi Arabia.

4.4.2 Hospital wastewater treatment

Onsite hospital wastewater treatment processes include three or four stages:

(1) Primary treatment to settle out larger suspended solids.

(2) Secondary treatment to reduce organic matter by biological treatment.

(3) Tertiary treatment.

(4) Chlorination process for disinfection.

Onsite treatment plants are operated by the Ministry of Health under the control and monitoring of the Ministry of Water and Electricity in order to assess the quality of the effluent through regular sampling and analysis of pre-treated effluents. The effluent is normally discharged into the sewer system (via underground pipe) to Riyadh's central WWTP.

There are many WWTPs around Riyadh that serve the city. However, the largest WWTP, that receives the inflow of hospital wastewater, is the Riyadh central WWTP, shown schematically in Figure 4.4.

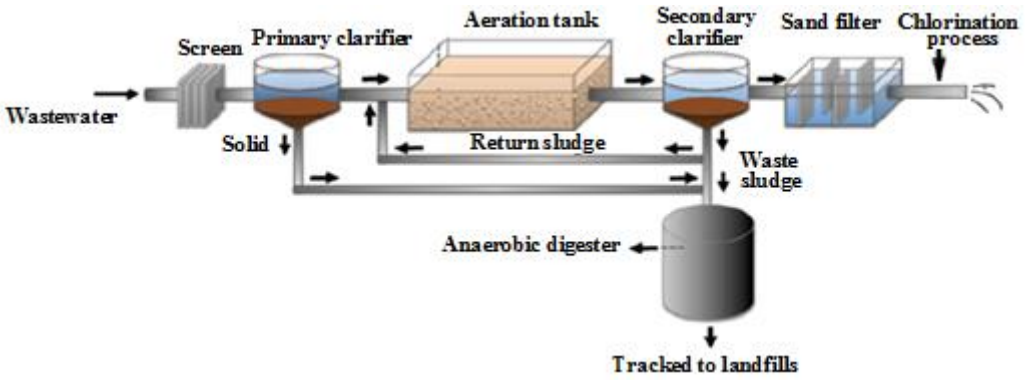

Fig. 4.4: Treatment schematics of a conventional wastewater treatment plant (Aljassim 2013)

It has a capacity of about 600,000 m^3/day and offers integrated advanced treatment, including preliminary treatment, primary treatment, secondary treatment (aeration tank) and tertiary treatment. Tertiary treatment is carried out using sand filtration and chlorination processes. The plant receives about 500,000 to 550,000 m^3 of wastewater daily (MOWE 2012). The flow rate tends to be higher during the summer due to increases in municipal water use as a result of the relatively high temperature.

4.4.3 Disposal of treated wastewater

All wastewater treatment plants must get a permit from the Ministry of Water and Electricity in order to discharge their treated effluent. The treatment plants are not allowed to discharge effluent to wells, dams and sources of drinking water (MOWE 2014). The effluent discharged must comply with minimum standards, as shown in Table 4.5 (MOWE 2012). The effluents from wastewater treatment plants can be used in irrigation, street cleaning and industrial applications.

Table 4.5: Maximum level of parameters permitted for discharge into the environment or system sewage (MOWE 2012).

Parameter	Maximum level
TSS	40 mg/L
pH	6-8.4
BOD_5	40 mg/L
Turbidity	5 NTU
phenols	0.002 mg/L
Coliform	1000 cells /100 ml
NO_3-N	10 mg/L
NH_3-N	5 mg/L

4.3 Conclusion

The UK and Saudi Arabia apply two different hospital wastewater management practices. A co-treatment with municipal wastewater is a common practice in the UK, while onsite treatment is common practice in Saudi Arabia. Furthermore, the treatment plants in both countries operate under different ambient temperature conditions. The next chapter will investigate the occurrence and performance of selected wastewater treatment plants in both countries in terms of removal of pharmaceutical compounds.

CHAPTER 5

Identification of key factors affecting pharmaceutical compounds removal in biological treatment under different environmental conditions

5.1 Introduction

The activated sludge process (ASP) is the most commonly used process in many municipal wastewater treatment plants (MWWTPs). The principal aim of ASP is to break down organic matter into carbon dioxide, water, and other inorganic compounds under aerobic conditions. A parameter commonly used for wastewater treatment design is the solid retention time (SRT) (Clara *et al.* 2005a; Fernandez-Fontaina *et al*. 2012). The SRT is related to the time of residence and growth rates of the microorganisms in the reactor that are able to reproduce during this time (Clara *et al.* 2005a). The maximum growth rates of microorganisms also depends on the temperature, where high temperatures enhance microbial activity and might be preferable for more effective removal (LaPara *et al*. 2000; Vieno *et al*. 2005; Massmann *et al*. 2006). The SRT, temperature and others factors, including the hydraulic retention time (HRT) (also known as the aeration time) and pH, have important effects on the development of slow-growth microorganisms, which subsequently influence the development of a high diversity of microorganisms in the reactors (Calderón *et al*. 2013). Microorganisms usually reach their optimal activity at warm temperatures of between 20 and 40°C (Kaleli and Islam 1997). The effects of temperature during the ASP have mainly been considered in studies that have

examined the microbial communities in laboratory experiments (Ahn and Logan 2010; Kaleli and Islam 1997) and have assessed seasonal variation (Calderón *et al*. 2013).

In this study, the fate of pharmaceutical compounds at four UK MWWTPs and two Saudi Arabian hospital WWTPs (HWWTPs) were assessed and considered in terms of the operational parameters employed under different environmental conditions: temperate climate (UK) and tropical climate (Saudi Arabia). The sampling regime and analytical methods employed in carrying out this study have been explained in Chapter 3.

5.2 Results and discussion

5.2.1 UK municipal wastewater treatment plants

5.2.1.1 Conventional parameters

The averages of the conventional parameters measured in the influent and effluent samples generally show high removal efficiencies in each MWWTP in the UK (Table 5.1) were measured. The average concentrations of BOD5, COD and TOC measured in the influents varied widely from plant to plant, being 45–191, 113–419 and 44–135 mg/L, respectively. The average concentrations of these parameters in the effluent were much lower, with BOD_5, COD and TOC ranges of 4–7, 19–30 and 7–10 mg/L, respectively (Table 5.1).

The Cupar, Guardbridge and Hatton plants (which use ASP) appeared to be much more efficient at removing organic matter than the Letham plant, which uses a TF.

Table 5.1: Influent and effluent concentrations of conventional parameters measured at municipal wastewater treatment plants in the UK.

	Letham (TF)			Cupar (ASP)			Guardbridge (ASP)			Hatton (ASP)		
	Inf. (mg/L)	**Eff.** (mg/L)	**RE** (%)	**Inf.** (mg/L)	**Eff.** (mg/L)	**RE** (%)	**Inf.** (mg/L)	**Eff.** (mg/L)	**RE** (%)	**Inf.** (mg/L)	**Eff.** (mg/L)	**RE** (%)
BOD_5	45±17	7±2	84	191±45	5±3	97	65±23	6±2	91	54±23	4±2	93
COD	117±38	30±6	74	419±114	22±5	95	150±46	19±6	87	113±42	23±4	80
TOC	44±13	10±3	77	135±46	7±2	95	52±29	10±3	81	49±18	8±2	84
NO_3	13±9	51±16	-	3±3	34±8	-	9±7	61±16	-	1±2	1±2	-
NH_4	11±6	0.7±0.7	94	24±11	0.3±0.5	99	21±10	0.1±0.2	100	22±7	19±6	14

Results are reported as mean ± standard deviation; Inf: Influent; Eff: Effluent; RE: Removal Efficiency; ASP: Activated Sludge Plant; TF: Trickling Filter Plant.

The performance of the nitrification processes in the Letham, Cupar and Guardbridge plants were efficient (they measured 94%, 99% and 100% removal efficiency, respectively), while in the Hatton plant there was no significant change in ammonium concentrations (Table 5.1). Elevated ammonia concentrations of 13–30 mg/L were observed in the effluent of the Hatton plant, while low average ammonia concentrations (<0.3 mg/L) were observed for both the effluent from the Cupar and Guardbridge MWWTPs. Nitrate concentrations for Cupar and Guardbridge plants varied between 18 and 75 mg/L confirming efficient biological ammonia removal.

In general, the removal efficiency of organic compounds (BOD_5, COD and TOC) was higher in the ASP than in the TF. In terms of the nitrifying conditions, the Cupar, Guardbridge and Letham plants were observed to be efficient, while poor nitrification processes were found to have occurred at Hatton. Based on the observations, the poor nitrification process is likely to be attributable to an insufficient aeration time.

5.2.1.2 Pharmaceutical compounds

Table 5.2 shows the concentrations of selected compounds in both influent and effluent samples from the four UK MWWTPs. Of the fifteen pharmaceuticals analysed in the wastewater samples, eight compounds were detected: antibiotics (sulfamethoxazole and its metabolite, N-acetyl-sulfamethoxazole), two analgesics (naproxen and paracetamol), one β-blocker (atenolol), one anaesthetic (lidocaine), one antidepressant (carbamazepine) and one lipid regulator (bezafibrate). One compound was detected but could not be quantified (clarithromycin), and the other compounds analysed including ibuprofen, ciprofloxacin, diatrizoate, iopamidol, cyclophosphamide and ifosfamide were not detected in the samples.

A high variation in the concentrations of the compounds examined was observed across the MWWTPs. This variation may have arisen due to various different factors such as consumption, excretion or uncertainty arising from the sampling methods. Uncertainty related to sampling may influence data accuracy in that the uncertainly may be due to the varied sampling times employed as discussed in Chapter 3 (page 53). In addition, the potential adsorption of pharmaceutical compounds onto sludge (this aspect was not investigated in the study) during the treatment processes can also influence the data accuracy between influent and effluent.

The results showed that the Hatton plant had relatively high concentrations of pharmaceutical compounds in the raw wastewater. This is likely to be because the plant receives wastewater from many hospitals, unlike the other three plants (see Table 3.3), which is likely to contribute to high levels of pharmaceutical compounds in the resulting wastewater.

Table 5.2: Concentrations of pharmaceutical compounds in selected municipal wastewater treatment plants in the UK.

		LOQ ng/L	Hatton		Cupar		Letham		Guardbridge	
Class	**Compound**		Inf. (ng/L)	Eff. (ng/L)	Inf. (ng/L)	Eff. (ng/L)	Inf. (ng/L)	Eff. (ng/L)	Inf. (ng/L)	Eff. (ng/L)
Analgesics and Anti-inflamma-tories	Paracetamol	100	7290	n/d	5248	n/d	3701	1068	3574	n/d
	Naproxen	200	2106	575	1023	<LOQ	n/d	n/d	2441	n/d
	Ibuprofen	1000	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Antidepressants	Carbamazepine	80	657	951	567	926	<LOQ	-	653	856
β-blockers	Atenolol	80	384	149	601	< LOQ	301	149	159	103
Lipid Regulators	Bezafibrate	200	1221	1121	494	232	n/d	n/d	1852	369
Anaesthetics	Lidocaine	80	193	217	n/d	206	n/d	n/d	n/d	148
Antibiotics	Ciprofloxacin	400	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	Clarithromycin	200	n/d	<LOQ	n/d	<LOQ	n/d	<LOQ	n/d	<LOQ
	Sulfamethoxazo.	80	n/d	132	n/d	n/d	n/d	n/d	n/d	n/d
Metabolite of sulfamethoxazo.	NACS	200	401	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Contrast Media	Diatrizoate	400	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	Iopamidol	400	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Cytostatics	Cyclophospham.	200	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	Ifosfamide	80	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

Inf = influent; Eff = effluent; Sampling period: 3.10.2012–9.11.2012 and 3.6.2013–28.6.2013 twice weekly (n = 1, composite samples made from the samples taken during both periods); LOQ = limit of quantitation (substances detected but not quantifiable); n/d = not detected.

Of the compounds that were detected in the influent samples, the concentrations of the over-counter drugs, paracetamol and naproxen, were highest from 3701 ng/L to 7290 ng/L for paracetamol and up to 2441 ng/L for naproxen. Bezafibrate was also detected at a concentration of 1852 ng/L in the raw influent of the Guardbridge plant. Hatton influent had the highest paracetamol concentration (7292 ng/L) and Guardbridge influent had the highest naproxen and bezafibrate concentrations at 2441 ng/L and 1852 ng/L, respectively. The concentrations of naproxen and bezafibrate in the influent samples were similar to results reported in the literature. For example,

Kasprzyk-Hordern *et al.* (2009b) reported naproxen concentrations of 510–2480 ng/L in the influents of two MWWTPs in Wales and concentrations of bezafibrate were measured at around 2000 ng/L by Miege *et al.* (2009). Furthermore, higher concentrations of paracetamol than those recorded in this study, of up to 180 µg/L, have also been detected in Spanish MWWTPs (Radjenovic *et al.* 2009) and Welsh MWWTPs (Kasprzyk-Hordern *et al.* 2009b). Lower concentrations of paracetamol, naproxen and bezafibrate were observed in the WWTP effluents, with maximum removal efficiencies of > 99% for paracetamol and naproxen (Figure 5.1). The removal efficiencies of the compounds varied between the MWWTPs. An almost complete removal of paracetamol and naproxen was achieved in the Guardbridge and Cupar plants, while the removal efficiency of naproxen in the Hatton plant was about 73%. The removal efficiencies of bezafibrate among the MWWTPs ranged from 8% (Hatton) to 80% (Guardbridge).

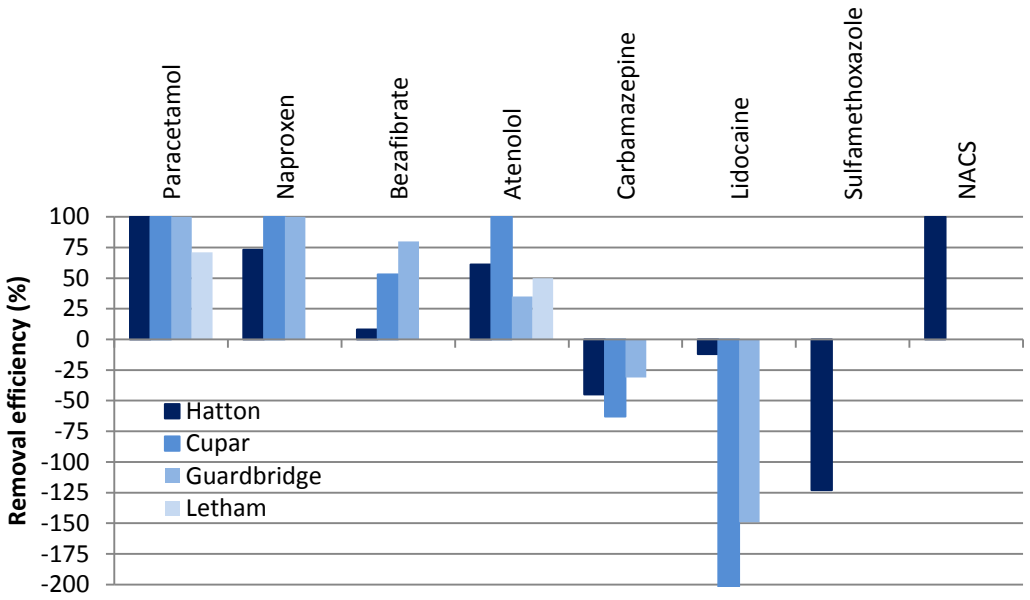

Fig. 5.1: Removal efficiencies of pharmaceutical compounds in municipal wastewater treatment plants in the UK.

Carbamazepine was detected in most of the MWWTP samples in this study. It did not exhibit large variations in concentration between the influents and effluents of the MWWTPs (567–926 ng/L). Removal of the compound was poor in all WWTPs, with higher concentrations (31- 63% higher) measured in the effluents than the influents at each ASP. Carbamazepine has been reported as recalcitrant to biological treatment processes and, thus, poor removal efficiencies have been found in respect of conventional biological treatment and membrane bioreactor (MBR) treatments (Jelic *et al.* 2012b; Rua-Gomez and Puttmann 2012). In addition, the compound has frequently been detected in WWTP effluents and river samples from European and North American waters by prior research studies (Ternes 1998; Heberer 2002; Metcalfe *et al.* 2003 and Miao *et al.* 2005). The carbamazepine results from this study are consistent with those presented in previous research, where carbamazepine concentrations in effluent samples were found to be 20–100% higher than in influent samples (Joss *et al.* 2005; Clara *et al.* 2005a and Vieno *et al.* 2007). The increases in carbamazepine concentrations may be attributed to its metabolic chemical properties. Carbamazepine is excreted from humans in the form of carbamazepine itself or as its metabolites, but during wastewater treatment processes the metabolites can be converted back to the parent compound through enzymatic processes (Vieno *et al.* 2007; Leclercq *et al.* 2009).

Atenolol was also found in most of the influents and effluents of the MWWTPs sampled in this study. The Cupar plant had the highest influent concentration, of 601 ng/L, while Guardbridge had the lowest influent concentration, at a level of 160 ng/L. In the effluents of the MWWTPs, the concentrations of atenolol

were found to be much lower, between 149 ng/L (Hatton) and at a concentration less than the limit of quantitation (LOQ) in the Cupar MWWTP.

Lidocaine was detected in most of the effluents of the MWWTPs, with concentrations ranging between 148 and 217 ng/L, but it was only detected in the influent samples of the Hatton plant. This discrepancy may be related to the metabolism processes, or to the instability of the compound (Rua-Gomez and Puttmann 2012). Lidocaine can be metabolized to monoethylglycinexylidide (MEGX) in humans (Tam *et al.* 1990). MEGX can further metabolize to 2,6-dimethylaniline (2,6-DMA) (Bangoluri *et al.* 2005). However, some metabolites can be converted back to their parent form during treatment processes (Watkinson *et al.* 2007). Therefore, it is possible that metabolites of lidocaine might be converted back to the parent compound. Rua-Gomez and Puttmann (2012) reported similar concentrations of lidocaine in Germany's WWTPs, ranging between 91 and 217 ng/L.

With regard to the antibiotics, while detected in the effluents, the concentrations of clarithromycin were found to be <LOQ. In contrast, concentrations of sulfamethoxazole and its metabolite, N-acetyl-sulfamethoxazole (NACS), were detected in the effluent and influent of the Hatton plant, respectively. Sulfamethoxazole was found at a relatively low concentration in the effluent (132 ng/L), while NACS was present at a higher concentration (402 ng/L). Normally, 50% of sulfamethoxazole is metabolized to NACS in the human body, while the residue remains unchanged (Gobel *et al.* 2005). During biological wastewater treatment processes, NACS can be converted back to its parent compound (Gobel *et al.* 2005; Gobel *et al.* 2007; Shelver *et al.* 2008). This may explain why NACS was detected in the influent and sulfamethoxazole was

found in the effluent. Ashton *et al*. (2004) reported similar results, in which NACS was observed at higher concentrations (33%) relative to sulfamethoxazole in the UK WWTPs.

5.2.1.3 Potential influences of the operational parameters on removal efficiencies

The removal efficiencies of the pharmaceutical compounds detected in the wastewater samples (Figure 5.1; Table 5.2), are discussed in this section in relation to the type of biological treatment and operational parameters employed. It is worth noting that only the elimination from the liquid phase was investigated in this study, and that it is possible that some of the micropollutants could have been adsorbed onto the sludge as reported in the Chapter 3.

- Paracetamol

Paracetamol was removed efficiently by all four of the MWWTPs. The MWWTPs that employed ASP (Hatton, Cupar and Guardbridge) achieved greater than 99% removal efficiencies, while 71% removal efficiency was observed in the Letham plant (which uses TF). These results correspond with those of Kasprzyk-Hordern *et al.* (2009b) and Verlicchi *et al.* (2013), who reported that ASP can achieve 99% removal efficiency for paracetamol. Overall, paracetamol was removed efficiently by the different MWWTPs using both processes (ASP and TF) under conventional operational parameters.

- Naproxen

The average removal efficiency of naproxen by the Guardbridge, Cupar and Hatton MWWTPs was 91%. The highest removal efficiencies of naproxen, which reached levels of over 99%, were observed in the Guardbridge and Cupar plants (nitrifying ASP), while the Hatton plant (non-nitrifying ASP) only achieved 73% removal efficiency (Figure 5.1). The high effectiveness of the nitrification processes during the biological treatment may be correlated with the HRTs which are longer in the Guardbridge and Cupar MWWTPs. From Table 3.2, the average operational HRTs for Cupar, Guardbridge and Hatton MWWTPs were 16, 17 and 4 hours respectively. Therefore, longer HRTs may have enhanced the biodegradation of naproxen at the Cupar and Guardbridge MWWTPs. Increases in the SRTs and HRTs can enhance bacterial growth and the establishment of diverse communities that have a greater capacity and potential for removing micropollutants (Kreuzinger et al. 2004). The results from this study compare favourably with the average removal efficiencies of 81% measured in six Taiwan WWTPs (Lin et al. 2010) and of 80% in WWTPs from five EU countries (Paxeus 2004). Lower average removal efficiencies of naproxen were also reported at several WWTPs, including 66% in Germany (Ternes 1998), 40–55% in Spain (Carballa et al. 2004) and 45% in Japan (Nakada et al. 2006).

- Bezafibrate

The removal efficiencies of bezafibrate varied across the MWWTPs, with an average of less than 50%; however, the removal efficiencies in the MWWTPs that used longer SRT and HRT were much higher (53–80%) than in the non-

nitrifying ASP (8%). Miege *et al*. (2009) and Kasprzyk-Hordern *et al*. (2009b) reported bezafibrate removal efficiencies of 60–70% during ASP. Furthermore, Castiglioni *et al.* (2006) reported that bezafibrate removal during ASP was variable and dependent on the season (they measured 15% and 87% removal efficiencies in winter and summer respectively). In this study, the higher bezafibrate removal efficiency obtained in the Cupar and Guardbridge MWWTPs was also likely to have been linked to their higher HRTs, when compared to the Hatton MWWTP.

- Atenolol

The average removal of atenolol among the four WWTPs was 62%. One of the nitrifying ASP (Cupar) was the most efficient at removal (> 99%), while the non-nitrifying ASP (Hatton) achieved a removal efficiency of 61%. The TF (Letham) only removed 50% of the atenolol. A recent study by Kasprzyk-Hordern *et al.* (2009b) found similar removal efficiency for atenolol in ASP (80%), when compared to TF processes (70%). It seems that atenolol is highly biodegradable, particularly in ASP with long HRTs.

- Lidocaine

Lidocaine was detected in the effluents (at levels of up to 260 ng/L) of the three ASPs, but only in the influent at Hatton MWWTPs and for Cupar and Guardbridge MWWTPs exhibited high negative removal efficiency for this reason (Figure 5.1). Consequently, removal efficiencies could not be calculated. Rúa-Gómez and Püttmann (2012) reported that lidocaine was only partially removed through different WWTPs. The concentrations detected in the

effluents of this study were below the PNEC available from the literature (82 µg/L, Verlicchi *et al.* 2012a); therefore, it is assumed no immediate risk was being posed to aquatic organisms. Nevertheless, the continuous discharge of lidocaine into receiving waters may lead to its accumulation, which could have long-term effects.

- Carbamazepine

As discussed above, carbamazepine concentration was found to have increased by up to 63% in the effluents when compared to the influents. This indicates that the ASPs may be ineffective at removing the compound for the range of SRT and HRT that the selected plants were operating at during the sampling period. Kasprzyk-Hordern *et al*. (2009b) also observed poor removal efficiencies of carbamazepine in ASPs in Wales. The range of carbamazepine removal efficiencies by MWWTPs, based on 19 published studies, averaged below 10% (Zhang *et al.* 2008). The widespread detection of carbamazepine in the environment may be related to its high persistence during conventional treatments at MWWTPs, where it is believed to neither degrade nor adsorb onto sludge (Clara *et al.* 2004). The concentrations detected in the effluents in this study (up to 951 ng/L) were below the PNEC available from the literature (13.8 µg/L, Verlicchi *et al.* 2012a). However, the continuous discharge of this compound to receiving waters may build up to levels of concentration that are toxic to aquatic organisms.

- Sulfamethoxazole and its metabolites (NACS)

Sulfamethoxazole was the only antibiotic detected in the effluent of the Hatton plant. Consequently, removal efficiencies could not be calculated and a high negative is shown in figure 5.1. NACS may have been removed or converted back to its parent compound (sulfamethoxazole) during treatment, as discussed above (page 86).

5.2.1.4 Discussion of the importance of key factors on removal efficiency

In MWWTPs, the elimination of pharmaceuticals is affected by many factors, such as temperature, SRT, HRT and the level of dilution (Kasprzyk-Hordern *et al.* 2009b; Verlicchi *et al*. 2012a). In this study, the samples collected during winter and summer were mixed together; therefore, the effect of temperature on the removal of the compounds cannot be assessed. However, the other factors are discussed below.

- HRT

Activated sludge processes, which employed longer HRTs in this study, were found to enhance the biodegradation rates of some compounds. These increases in removal efficiencies were likely due to the longer available adaption and development period for certain microbial communities that are able to break down pharmaceuticals (Falas et al. 2012; Clara et al. 2005a). For example, the Guardbridge and Cupar MWWTPs used longer HRTs and achieved more effective removal of some compounds (e.g., naproxen,

bezafibrate and atenolol), in comparison to the Hatton MWWTP, which had a poor nitrifying process and a shorter HRT. Hence, effective nitrification can be used as an indicator of the length of biodegradation times. That is, the longer the HRT the greater the potential for effective nitrification, and the longer the period for microbial adaptation and biodegradation of pharmaceutical compounds. These results are in accordance with those of other studies (Fernandez-Fontaina et al. 2012; Clara et al. 2005a; Estrada-Arriaga et al. 2011), which reported positive correlations between the removal rates of several pharmaceuticals and the SRT and/or HRT.

- Dilution of wastewater

The dilution factor, for example by heavy rainfall or high flow rates, may reduce the influent concentrations and may also deteriorate the removal efficiencies of pharmaceutical compounds (Helwig *et al.* 2013; Joss *et al.* 2006). Also, the entry of excess water may reduce the HRT in the treatment plant (Vienoa *et al.* 2007; Vienoa *et al.* 2005). The deterioration of removal efficiencies in these situations has been linked to decreases in the biodegradation rates of the pharmaceuticals (Joss *et al.* 2006). In this study, a period of heavy rain occurred during the sampling period in winter, especially in Hatton area. This might have caused a decrease in the HRT used by the MWWTP (data not available). Therefore, the concentrations of compounds in the samples from this period and their removal efficiencies may have been influenced by the dilution factor. However, the effects of dilution would have also reduced the possible environmental hazards posed by the compounds to aquatic organisms, especially in terms of the acute toxicity or chronic effects (Gros *et al*. 20101).

5.2.2 Saudi Arabia hospital wastewater treatment plants

5.2.2.1 Conventional parameters

Table 5.3 summarises the average concentrations of conventional parameters for the influent and effluent samples from both of the hospitals chosen in this study. The average concentrations of COD and nutrients in the influents of the HWWTPs were found to be similar. The average COD observed in the influents of the Salman HWWTP (SHWWTP) and the Imam HWWTP (IHWWTP) were 376 and 336 mg/L, respectively. The COD in the effluents were much lower, at 64 and 27 mg/L, respectively. However, the COD of the effluent from the SHWWTP was relatively higher than the COD limit values of restricted irrigation in Saudi Arabia (Chapter 4; Table 4.4) as set out in (MOWE 2014).

Table 5.3: Influent and effluent concentrations of conventional parameters measured at hospital wastewater treatment plants in Saudi Arabia.

Parameters	**SHWWTP**			**IHWWTP**		
Process technology	Aerobic			Aerobic		
Ambient temperature (ºC)	28±7			28±7		
pH	7.0-7.5			6.8-7.5		
	Inf. (mg/L)	**Eff.** (mg/L)	**RE** (%)	**Inf.** (mg/L)	**Eff.** (mg/L)	**RE** (%)
COD	376	64	83	336	27	92
NH_4	22.3	0.26	99	18.97	0	100
NO_2	0.30	0.61	-	0.33	0.25	-
NO_3	0.23	0.67	-	0.35	2.43	-

SHWWTP = Salman hospital wastewater treatment plant; IHWWTP = Imam hospital wastewater treatment plant; ER= Removal Efficiency

The performance of the nitrification process in both HWWTPs was observed to be efficient (Table 5.3); the average ammonia concentrations observed in the effluents from both HWWTPs were less than 0.3 mg/L. Data on the HRTs or

SRTs were not available, but highly efficient nitrification processes may be influenced by the high temperatures in Saudi Arabia, as normally, the activity of nitrifying bacteria increases with temperature (Kaleli and Islam 1997). The low concentrations of nitrate in the effluents, despite the almost 100% removal of influent ammonia suggests that the operational methods of the plants might have encouraged significant denitrification (i.e., nitrate conversion to nitrogen gas) to occur. It is therefore assumed, in the interpretation of the results, that the plants were operated at HRT and SRT values that enabled effective nitrification to take place.

5.2.2.2 Pharmaceutical compounds

Table 5.4 summarises the mean concentrations of the selected compounds in the influent and effluent samples obtained from the two hospital wastewater treatment plants (HWWTPs). Nine pharmaceutical compounds were detected out of the twelve compounds analysed, which included four antibiotic compounds (ciprofloxacin, clarithromycin, sulfamethoxazole, and its metabolite NACS), one analgesic (paracetamol), one stimulant (caffeine), one β-blocker (atenolol), one anaesthetic (lidocaine) and one antidepressant (carbamazepine). The other compounds tested for, including bezafibrate, erythromycin and cyclophosphamide, were not detected in either the influents or effluents of the HWWTPs. Caffeine and paracetamol were detected in all the influent samples and were present at the highest concentrations of all the compounds analysed, at 25828–91593 ng/L and 12008–12673 ng/L, respectively. Especially high concentrations of caffeine (> 90 µg/L) were found at the SHWWTP influent. Caffeine has been detected in MWWTPs around the

world ranging from 3.4–6.6 µg/L in China (Sui *et al.* 2010), to 7–73 µg/L in Swiss MWWTPs (Buerge *et al.* 2003) and at concentrations up to 89 µg/L in Spanish MWWTPs (Martín *et al*. 2012).

Table 5.4: Concentrations of pharmaceutical compounds in hospital wastewater treatment plants in Saudi Arabia (ng/L)

Class	Compound	LOQ	SHWWTP Inf.	SHWWTP Eff.	IHWWTP Inf.	IHWWTP Eff.
Analgesics	Paracetamol	0.5	12390±343	73±11	12303±177	157±20
Antidepressant	Carbamazepine	0.25	151±13	41±1	73±14.3	n/d
β-blockers	Atenolol	5.0	728±82	46±2	329±27.9	55±3.6
Lipid	Bezafibrate	0.1	n/d	n/d	n/d	n/d
Anaesthetics	Lidocaine	1.0	158±12	114±4	129±6	<LOQ
Antibiotics	Ciprofloxacin	2.5	5611±657	n/d	2183±251	n/d
	Clarithromycin	0.5	83±72	22±9	38.0	n/d
	Sulfamethoxazol	1.0	30±7	n/d	132±5	n/d
	Erythromycin	0.5	n/d	n/d	n/d	n/d
Metabolite of sulfamethoxaz.	NACS	5.0	1234±55	59±14	506±21	n/d
Cytostatic	Cyclophosphamide	1.0	n/d	n/d	n/d	n/d
Others	Caffeine	2.5	74794±15502	n/d	27469±2018	n/d

Results are reported as mean ± standard deviation (n= 3); LOQ = limit of quantitation (substances detected but not quantifiable); n/d = not detected.

The high concentrations of caffeine observed in this study may be related to its administration along with other medication in order to enhance the effects of certain analgesics in cough, cold, and headache medicines (Lin *et al.* 2010; Weigel *et al.* 2002). It is also used as a cardiac, cerebral and respiratory stimulant and as a diuretic (Buerge *et al.* 2003). Both caffeine and paracetamol were almost completely removed from both of the HWWTPs. Negligible concentrations of paracetamol were detected in the HWWTP effluents, while

the removal efficiencies for caffeine were near 100%. Alidina *et al.* (2014) reported high concentrations of caffeine (64–16500 ng/L) in the effluent of six Saudi MWWTPs (but no data were available for influent concentrations in their study).

As expected, the hospital influents were found to be significant sources of antibiotics in this study, and four of the antibiotic compounds examined (ciprofloxacin, clarithromycin, sulfamethoxazole and NACS) were detected at concentrations ranging from 30 to 5611 ng/L in the influents of the HWWTPs. The high concentration of the antibiotics in the raw HWW is likely to be due to the high levels of antibiotic consumption in hospitals (Kümmerere 2009). Ciprofloxacin was present in both influents at the highest concentrations of the antibiotic compounds tested, followed by NACS (a metabolite of sulfamethoxazole), with ranges of 2017–6105 ng/L and 483–1288 ng/L, respectively. Relatively low concentrations (<160 ng/L) of sulfamethoxazole and clarithromycin were detected in the raw HWW. It has previously been reported that high concentrations of antibiotics in effluents are of particular concern due to the potential for genotoxic effects and the development of antibiotic resistance in the environment (Brown *et al.* 2006). The spread of antibiotic resistance is a major threat to public health (Rogues *et al.* 2004; Vander Stichele *et al*. 2006). Previous studies have also found high concentrations of sulfamethoxazole (730 ng/L) in Saudi effluents from MWWTPs (Alidina *et al*. 2014). However, in this study the concentrations of all the antibiotics in the effluents of the HWWTPs were found to be negligible, and lower than their respective PNECs reported in the literature (Verlicchi *et al.* 2012a).

Atenolol, carbamazepine and lidocaine concentrations were consistently detected in the influent samples of the HWWTPs at relatively low concentrations, of 304–824, 64–160 and 122–166 ng/L, respectively. Previously, atenolol was detected at low concentrations (1–4 ng/L) in the influent of a Saudi MWWTP (Shraim *et al.* 2012). These drugs are relatively recalcitrant to biological treatment and are generally only partially removed in wastewater treatment systems. In this study, negligible concentrations of these drugs were detected in the effluents of the HWWTPs, at 44–58 ng/L, up to 41 ng/L and up to 118 ng/L, for atenolol, carbamazepine and lidocaine, respectively. These concentrations are lower than their respective PNECs (Verlicchi *et al.* 2012a). It should be noted, though, that a previous study has reported higher concentrations of atenolol (15–2550 ng/L) and carbamazepine (57–1200 ng/L) in the effluents of various MWWTPs in Saudi Arabia (Alidina *et al.* 2014).

5.2.2.3 Potential influence of the operational parameters on removal efficiencies

The removal efficiencies of pharmaceutical compounds from the HWW samples show that, on average, the most efficiently removed compounds were ciprofloxacin, caffeine, sulfamethoxazole, paracetamol and NACS (> 95%), followed by atenolol, carbamazepine and clarithromycin (> 85%) (Figure 5.2). The average removal efficiency of lidocaine was measured as greater than 65% at the two HWWTPs.

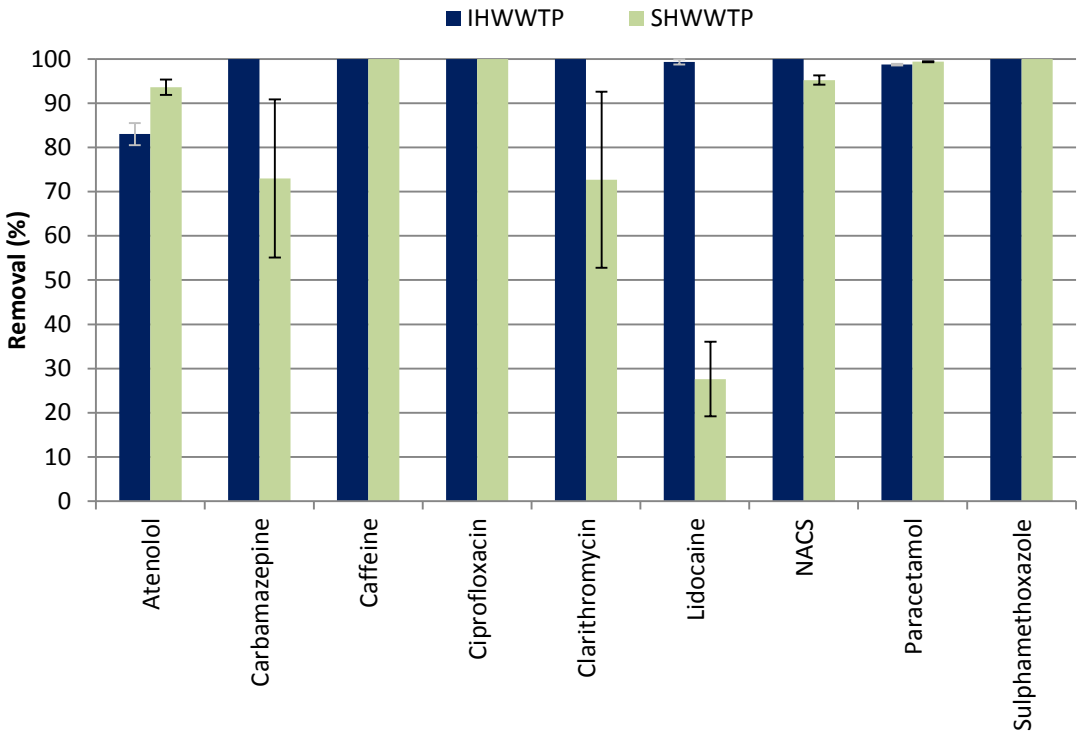

Fig. 5.2: Removal efficiencies of pharmaceutical compounds at the hospital wastewater treatment plants in Saudi Arabia. Results shown are mean ± standard deviation (n = 3).

- Paracetamol and caffeine

Caffeine and paracetamol were both almost completely removed from each of the HWWTPs. The average removal efficiency of paracetamol was greater than 98%, while for caffeine it was completely (100%) removed by both HWWTPs. Similar results were obtained for the removal of paracetamol (up to 99%) in ASP as found by Kasprzyk-Hordern *et al*. (2009b) and by Verlicchi *et al.* (2013), under temperate climate conditions. Thus, paracetamol removal at WWTPs seems possible with conventional ASP under different climate conditions (in both cold and warm weather). With regard to the removal of caffeine, Lin *et al.* (2010) reported similar caffeine removal efficiencies (99%) in six WWTPs in Taiwan. However, in contrast to these results, Buerge *et al.* (2003) found that

the removal efficiencies of caffeine varied (81–99%) at 13 Swiss MWWTPs. The authors indicated that the MWWTPs that were less efficient at removing caffeine (81%) had lower adaptation times for the microorganisms in the AS system (< 5 days versus > 5 days). As mentioned before, bacterial adaptation in ASP is enhanced through longer SRTs and higher temperatures (Batt *et al.* 2006). Therefore, the high ambient temperatures and the possibility that both plants may have been operated at HRTs and SRTs that encouraged effective nitrification may have provided the conditions the enabled the effective biodegradation of both compounds.

- Antibiotics

The mean removal efficiencies of ciprofloxacin, clarithromycin, sulfamethoxazole and NACS at the SHWWTP were 100%, 87%, 100% and 98%, respectively. The IHWWTP achieved 100% removal of all the antibiotics. Lin *et al.* (2009) previously studied sulfamethoxazole and clarithromycin, and reported that the removal efficiencies of six different WWTPs in Taiwan were greater than 50% and 20%, respectively. Carballa *et al.* (2004) also found a lower level of removal efficiency (< 60%) of sulfamethoxazole in ASP under temperate climate conditions (Galicia, Spain). A seasonal variation in the removal of sulfamethoxazole was observed, with a higher removal rate in summer (71%) than that which was observed in winter (17%) in Italy (Castiglioni *et al.* 2006). These results are much lower than the results observed in this study. Other studies indicated that re-conjugation of its metabolite (NACS), and thus higher concentrations of sulfamethoxazole occur in effluent compared to influent (Gobel *et al.* 2007; Shelver *et al.* 2008; Ashton *et al.* (2004). In this

study high removal efficiencies (> 98%) of both sulfamethoxazole and NACS were observed under tropical climate conditions and no concentrations of sulfamethoxazole were detected in the effluent samples. Consequently, no concentrations of NACS were left to re-conjugate to parent compounds as had been observed in the UK samples (page 86).

The other antibiotic (ciprofloxacin) was also completely removed (100%) in both HWWTPs. Gao *et al.* (2012) reported a lower removal efficiency (67%) in a Chinese ASP. Ciprofloxacin is a fluoroquinolone and it is known that adsorption to sludge is a major removal process. For example, a significant amount of ciprofloxacin (up to 90%) was removed via adsorption when the pH was less than 5.5 in a laboratory experiment (Githinji *et al.* 2011). However, adsorption has been observed to decrease with increasing temperatures (Paus *et al*. 2014) and an increase in pH (pH > 6) (Githinji *et al.* 2011). In this study, the removal efficiency of ciprofloxacin occurred under a high ambient temperature (>26^{o}C) and normal pH (7–8) conditions, which indicates that its removal appeared to be more as a result of biodegradation than adsorption.

In summary, the high removal efficiencies of antibiotic compounds observed in this study may have occurred due to the high ambient temperatures and sunlight under the tropical climate conditions (in Saudi Arabia). This is because the high temperatures, in the range of 25 to 45°C, are likely to have promoted the growth rates and yields of the bacterial communities (LaPara *et al*. 2000).

- Carbamazepine

The removal efficiency of carbamazepine by WWTPs has previously been found to be poor (Clara *et al.* 2004; Mohapatra *et al.* 2012), mostly below 10%

(Zhang *et al.* 2008). Furthermore, an increase in carbamazepine concentrations after wastewater treatments has been reported (Joss *et al.* 2005; Vieno *et al.* 2007) and was also observed in this study in relation to the UK MWWTPs (Table 5.2). In the treatment plants in Saudi Arabia as found in this study, a high average removal efficiency of carbamazepine (> 87%) at both HWWTPs was observed. The IHWWTP achieved 100% removal of the compound. In Malaysia, Dordio *et al.* (2009) has reported similar removal efficiencies in a constructed wetland (CW) in summer (97%), but at a lower level in winter (88%). The high ambient temperatures in Saudi Arabia may also have played a role in the higher removal efficiencies observed for carbamazepine. These findings were unexpected; they suggest that conventional WWTPs could remove carbamazepine under the right conditions and that tropical climate conditions are more favourable than temperate ones.

- Atenolol

The average removal efficiency of atenolol by the HWWTPs was 89%. The removal efficiencies of this compound reported in the literature vary drastically from study to study. For example, in WWTPs located in a temperate climate in Europe, Paxeus (2004) reported removal efficiencies of 10%, while Vieno *et al.* (2005) reported a removal efficiency of 61%. Castiglioni *et al*. (2006) found that the removal efficiency of atenolol was affected by temperature, where higher removal efficiencies were achieved in summer (55%) than in winter (10%). This indicates that the high removal efficiencies achieved by the HWWTPs in Saudi Arabia observed in this study could be due to higher microbial activity in a tropical climate.

- Lidocaine

The average removal efficiency of lidocaine by the HWWTPs was 64%. The IHWWTP achieved 100% removal. The removal efficiency of lidocaine was tested in various WWTPs in a temperate climate (Hesse, Germany), where it was found to be significantly lower (10–50%) (Rúa-Gómez and Püttmann 2012) than that observed in this study.

5.2.2.4 Discussion of the importance of key factors on removal efficiency

The removal efficiencies of the pharmaceutical compounds measured in this study appeared to be much improved in the hotter, tropical climate of Saudi Arabia. The respective effects of key factors on the elimination of pharmaceuticals in the studied WWTPs are now discussed below:

- Dilution factor

The high dilution of raw sewage has been reported to reduce the removal efficiencies of pharmaceutical compounds during sewage treatment (Vieno *et al.* 2007). In this study, no dilution of the raw sewage had occurred (that is, the HWW was treated onsite, and not combined with rainwater, or other wastewater sources). Therefore, the higher removal efficiencies of the pharmaceutical compounds could also be attributed to a lack of dilution. For example, caffeine, paracetamol and ciprofloxacin were found in high concentrations in the influents to the HWWTPs, which probably then allowed higher removal rates. These results suggest that the lower the dilution factor of raw wastewater, the greater the removal efficiency. However, in the case of other compounds (e.g.

carbamazepine, atenolol, lidocaine and sulfamethoxazole) there were lower influent concentrations and yet higher removal efficiencies were still achieved. This indicates the possibility that other parameters, such as temperature, may affect the removal rates.

- Temperature

Higher removal efficiencies were observed during the summer in temperate climates, by an average of 25%, compared to winter (Vieno *et al.* 2005). In a previous study examining six large WWTPs in Italy, Castiglioni *et al.* (2006) also found higher removal efficiencies in summer (18.6°C) than in winter (9.7°C), except for carbamazepine and ciprofloxacin removal which were similar across the two seasons. In this study, 100% removal efficiencies in relation to carbamazepine and ciprofloxacin were achieved by the two HWWTPs. In addition, very high removal efficiencies with regard to antibiotics, atenolol and lidocaine were achieved; these compounds are normally found to be persistent at conventional WWTPs in temperate climates (Paxeus 2004; Rúa-Gómez and Püttmann 2012; Carballa *et al.* 2004). Thus, the higher ambient temperatures (>26°C) that are present almost year-round in the tropical Saudi Arabian climate may have enhanced the removal efficiencies of these compounds. The tropical conditions may have led to a high level of biological activity in the ASP, which may in turn have increased the biodegradation kinetics. Microorganisms living in reactors at WWTPs usually reach their optimal activity rates at warm temperatures, between 25–35°C (Cruikshank & Gilles 2007; Kareem 2013).

Other factors, like nitrifying bacteria and sunlight availability (which is important in photodegradation) may also influence the removal efficiency of

pharmaceutical compounds (Kasprzyk-Hordern *et al.* 2009a). However, more comprehensive studies are needed to simultaneously investigate these factors and the potentially interrelated effects of temperature variation on the removal of pharmaceutical compounds during biological treatment processes.

- Tertiary treatment

HWWTPs applied tertiary treatment in the forms of sand filtration and disinfection. It is possible that sand filtration had an effect on the efficiency of removal of the pharmaceutical compounds. However, the removal of pharmaceutical compounds during sand filtration has generally been reported to be inefficient (Hollender *et al.* 2009; Nakada *et al.* 2007). In this study, samples were collected from only raw sewage and final treated. In the interpretation of the results reported in this study, it has been assumed that sand filtration and chlorination played a negligible role in the fate of the target micropollutants in the plants. Therefore, the contribution of both unit operations to the removal of pharmaceutical compounds requires investigation.

5.3 Conclusion

The removal efficiencies of pharmaceutical compounds were investigated in various biological treatment systems under different environmental conditions in a temperate climate (UK) and a tropical climate (Saudi Arabia). A summary of the removal efficiencies achieved is presented in Table 5.5 and ranges from no removal to very high removal (with the following categories; no elimination, poor <30%, moderate 30-70%, high 70-90% and very high > 90%). The degree of

removal efficiency of the pharmaceutical compounds appeared to strongly depend on the wastewater technology implemented in the WWTPs and the environmental conditions.

Table 5.5: The average removal efficiencies of pharmaceutical compounds in temperate (UK) and tropical (Saudi Arabia) climates.

Class	Compound	Temperate climate (UK)	Tropical climate (SA)
Analgesics & anti-inflammatories	Naproxen	very high	n/i
	Paracetamol	very high	very high
Antidepressant	Carbamazepine	no elimination	high
β-blockers	Lidocaine	no elimination	high
Lipid	Bezafibrate	Moderate	n/d
Anaesthetics	Atenolol	moderate	high
Antibiotics	Ciprofloxacin	n/d	very high
	Clarithromycin	<LOQ	high
	Sulfamethoxazol	poor	very high
Metabolite of sulfamethoxazole	NACS	very high	very high
Others	Caffeine	n/i	very high

<LOQ= limit of quantitation; n/d= not detected; n/i=not included in the list

In general, high removal efficiencies of up to 100% of analgesics, anti-inflammatories and caffeine were achieved by most of the WWTPs, under both climatic conditions. Other compounds (carbamazepine and lidocaine) were not removed efficiently in the temperate climate, while high removal efficiencies were achieved in the tropical climate. Moderate removal efficiencies were achieved for bezafibrate and atenolol in the temperate climate, while high levels were achieved for atenolol in the tropical climate. Some of the antibiotic drugs

detected were not quantifiable in the UK WWTPs (except for sulfamethoxazole). This may be due to dilution effects or perhaps its consumption may be relatively low in the contributing catchment. In contrast, in the Saudi HWWTPs, antibiotic drugs were detected because hospitals are a point source for these compounds and there was low dilution of the HWW (on-site treatment and arid conditions). Very high removal efficiencies for most antibiotics, of up to 100%, were observed in the Saudi WWTPs.

The observed removal efficiencies varied widely for the different compounds likely due to the different types of plant, operational differences (e.g. treatment, SRT, HRT, etc), temperature and dilution effects. Effective nitrification activities (which is usually associated with relatively high SRTs), in this study, have been positively correlated with increased HRT biodegradation rates of some compounds (e.g. naproxen, bezafibrate and atenolol). In tropical climate, with high ambient temperatures, sunlight and arid conditions, the WWTPs achieved even higher degrees of removal efficiencies (on average, 90%), for the most common compounds, including carbamazepine, lidocaine and sulfamethoxazole which in contrast, were found to be poorly degraded in the temperate climate.

Overall, it can be hypothesized that longer SRT, HRT, an elevated ambient temperature, and dilution are the major factors that influence the removal efficiencies of pharmaceutical compounds. Ultimately, these conditions in ASP maximise the growth rate of microorganisms. However, it has to be emphasised that the removal efficiency of pharmaceutical compounds from wastewater during treatment does not necessarily indicate that they are fully degraded. Some compounds may only undergo partitioning from wastewater and be

adsorbed onto the sludge, without their complete mineralisation. Further treatment of the sludge may be then required in order to prevent the release of these compounds into the wider environment.

CHAPTER 6

Assessment of the biodegradation of selected pharmaceutical compounds under aerobic and anaerobic conditions

6.1 Introduction

There are two main processes that act simultaneously to remove pharmaceutical compounds during biological treatment; biodegradation by bacterial communities and adsorption onto the sludge (Rattier *et al.* 2012). Biodegradation is the most important process involved in the removal of most pharmaceutical compounds during the biological treatment. The removal efficiencies of the pharmaceutical compounds vary from partial biodegradation to complete mineralisation; the degree of the elimination process depends on factors including the behaviour of the compounds, the solids retention time (SRT), hydraulic retention time (HRT), temperature and biomass concentration, among others (Chapter 5). Operational parameters like the SRTs and HRTs are important to consider when designing wastewater treatment plants (WWTPs), because they can be optimised to encourage the growth of microorganisms (Fernandez-Fontaina *et al.* 2012). The development of a complex species structure of biomass in a biological treatment system is expected to have an impact on the elimination of micropollutants. Clara *et al.* (2005a) identified a critical SRT of 10 days for activated sludge process for the removal of common macro-organic compounds, but there are inadequate data and the literature is lacking, on the relationship between the SRT and the removal of micropollutants during WWTP. In addition, pharmaceutical compounds in some WWTPs

systems and landfills will encounter anaerobic conditions. For example, compounds adsorbed onto solid sludge, produced from activated sludge processes, are commonly treated under anaerobic conditions for stabilisation and biogas production. This is how solid sludge from the Hatton MWWTP in the UK is treated. A drawback of the adsorption of pharmaceutical compounds onto solid sludge is that the compounds can later be released into the environment through the transport of sludge and its application in agriculture (Clarke and Smith 2011). Furthermore, where the wastewater has to travel long distances in sewers to the points of treatment, the wastewater can become septic due to anaerobic conditions within the sewerage system. This condition is more pronounced in tropical countries, due to relatively high ambient temperatures. Therefore, aerobic and anaerobic biodegradation experiments are necessary in order to gain an insight into the biodegradation behaviour of our target pharmaceuticals under these conditions.

6.2. Materials and methods

The materials and methods of the biodegradation experiments are reported in Chapter 3 (section 3.6). Briefly, short-term batch biodegradation tests were conducted for a subset of the target pharmaceutical compounds (paracetamol, naproxen, ibuprofen and sulfamethoxazole) from the WWTP experiments. High levels of these compounds have been reported in wastewater and receiving water samples (Roberts and Thomas 2006; Ashton *et al.* 2004; Thomas and Hilton 2004). The selection of these compounds was also based on:

1. Their general use; the pain killers paracetamol, naproxen and ibuprofen are commonly used drugs and can be bought over the counter.
2. Earlier results from field studies (Chapter 5) showed paracetamol and naproxen to be present at high concentrations in the UK samples, and paracetamol to be present at high concentrations in the Saudi Arabia HWWTP samples.
3. Antibiotics are used extensively by humans to treat microbial infections. Sulfamethoxazole was the only antibiotic detected in samples from both the UK and Saudi Arabian studies. Therefore, it was selected as a representative for antibiotics.
4. Their potential impacts: there are relatively low PNECs for these compounds as reported in the literature review (Table 3.1).

6.3 Results and discussion

6.3.1 Aerobic biodegradation of selected compounds

The aerobic batch tests were performed in duplicate. The experiments were run for 15 days until the first compound was almost completely removed (in this case, it was the culture containing paracetamol as shown in Figure 6.1). Only paracetamol showed high biodegradation efficiency, averaging 98% over 15 days. At 10 days (the critical SRT time suggested by Clara *et al.* 2005a), the degradation efficiency of paracetamol was greater than 80%.

Yu *et al.* (2006) also observed almost complete biodegradation of paracetamol at different initial concentrations (1 µg/L and 50 µg/L) in aerobic batch experiments following 14 days of incubation at room temperature (25°C). In

addition, in samples collected from the various WWTPs (as explained in Chapter 5), the concentrations of paracetamol were almost completely removed in the activated sludge plants. These results suggest that paracetamol can be regarded as relatively readily biodegradable under aerobic conditions and is therefore unlikely to persist during biological treatment processes.

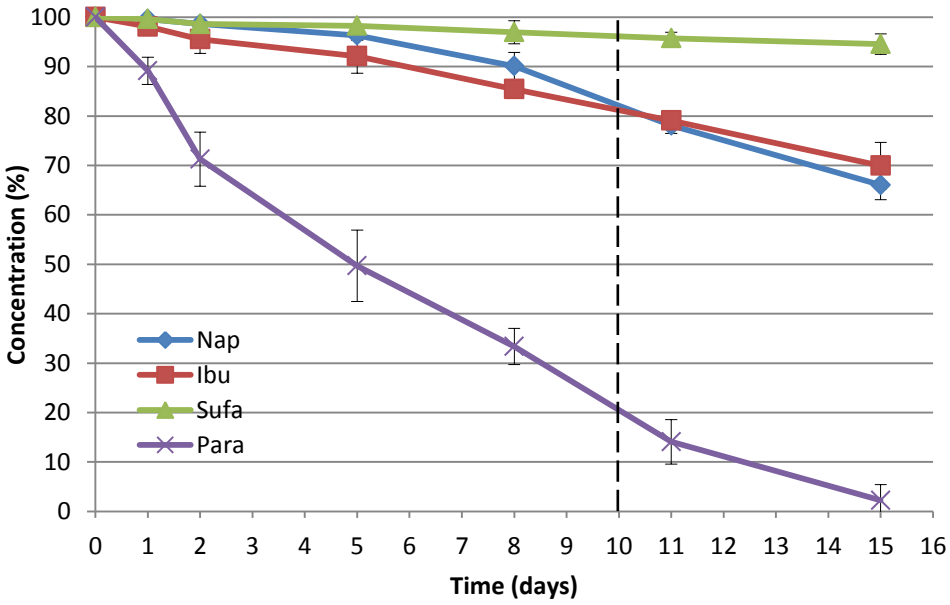

Fig. 6.1: Aerobic batch biodegradation of naproxen (Nap), ibuprofen (Ibu), sulfamethoxazole (Sufa) and paracetamol (Para) at various solid retention times. Results shown are mean ± standard deviation (n = 2); the vertical line represents a solids retention time of 10 days and allows comparison to the anaerobic digestion batch test.

The biodegradation rates of ibuprofen and naproxen were, relative to paracetamol, low (Figure 6.1). Their concentrations only decreased by 30% and 35% respectively over 15 days. Thus, the results suggest that ibuprofen and naproxen are not readily biodegradable. It can be seen from this figure that the degradation of naproxen started after a lag phase of about 5 days, after which point the concentrations of the compound started to decrease relatively quickly.

Grenni *et al.* (2013) also reported a lag phase, showing that the degradation of naproxen started after about 20 days. In the WWTPs study (Chapter 5), a high removal efficiency of naproxen was achieved at the nitrifying ASPs when a long aeration time was employed. Thus, the slow biodegradation of ibuprofen and naproxen in the batch tests study may be due to the bacteria present, which may not have adapted sufficiently to these pharmaceutical compounds during the experimental period. Additionally, there may have been limited adsorption of the compounds onto the culture biomass, since the solids contents of the culture bottles were relatively low. Nevertheless, adaptation of the bacterial biomass during WWTP processes has been identified as highly beneficial to compound biodegradation and this can be enhanced with longer SRTs in activated sludge (Falas *et al.* 2012; Petrie *et al*. 2014).

There was no decrease in the concentration of sulfamethoxazole in the aerobic tests over the 15 day experimental period as shown in Figure 6.1. In addition, no significant influence of SRT was observed in the removal of sulfamethoxazole during the tests. Other studies have also shown that sulfamethoxazole is not readily biodegradable under aerobic conditions (Al-Ahmad *et al*. 1999; Alexy *et al*. 2004; Gartiser *et al*. 2007). In addition, Larcher and Yargeau (2011) investigated the biodegradation of sulfamethoxazole by cultures of individual species of bacteria and a culture that included seven selected aerobic species of bacteria. The *Rhodococcus equi* culture resulted in 29% removal of sulfamethoxazole, while the culture with seven species of bacteria only removed 5% of the compound (the initial sulfamethoxazole concentration was 6 mg/L). It has also been reported that aerobic biomass must be highly acclimated in order to efficiently remove sulfamethoxazole (Drillia *et*

al. 2005). The results of the aerobic tests support these conclusions and indicate that SRTs greater than 15 days may be required to improve biodegradation rates.

6.3.2 Anaerobic biodegradation of selected compounds

The biodegradation of the selected pharmaceutical compounds (paracetamol, naproxen, ibuprofen and sulfamethoxazole) under anaerobic conditions was assessed over 10 days until the first compound was almost completely removed (in this case, it was the culture containing sulfamethoxazole that showed more than 98% removal on Day 10 as shown in Figure 6.2). The elimination rate of sulfamethoxazole was consistent over the period. It is clear that sulfamethoxazole was biodegraded much faster under anaerobic conditions than in aerobic conditions (Figure 6.1). These results are consistent with those of Carballa *et al.* (2007) who found that the percentage removal of sulfamethoxazole under anaerobic conditions was about 98% over 10 days, at different initial concentration levels (4–400 µg/L). A field study also found that the biodegradation of sulfamethoxazole that occurred in anaerobic sludge removed more than 90% of the compound (Narumiya *et al.* 2013). Overall, the findings of this study support those of others and suggest that anaerobic conditions can efficiently eliminate sulfamethoxazole (and possibly other antibiotic compounds).

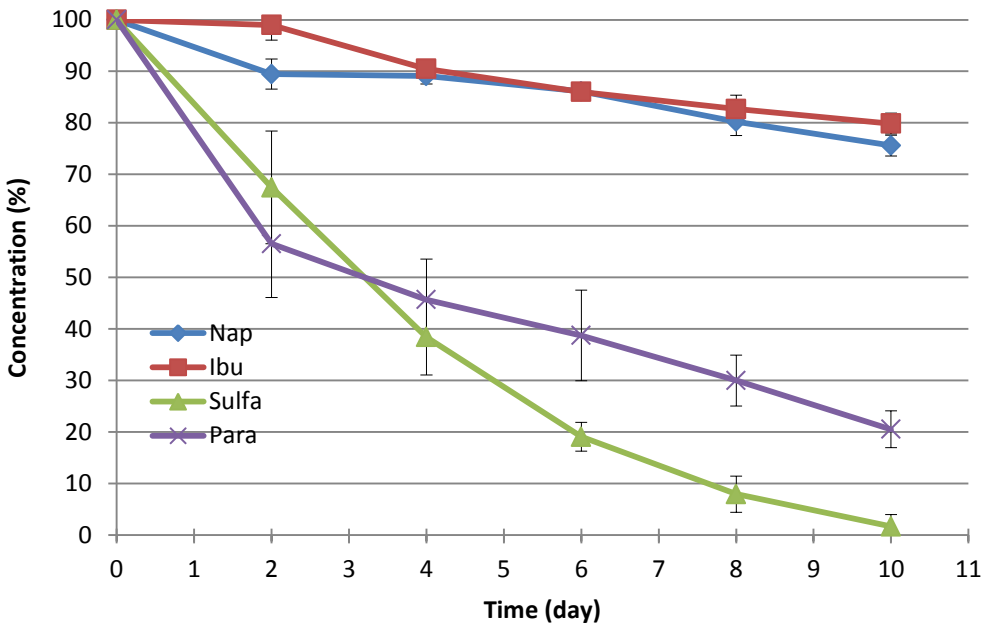

Fig. 6.2: Anaerobic batch biodegradation of naproxen (Nap), ibuprofen (Ibu), sulfamethoxazole (Sufa) and paracetamol (Para) at various solid retention times. Results shown are mean ± standard deviation (n = 2).

Figure 6.2 also shows that paracetamol decreased by 80% over the 10-day period. It decreased by about 45% in the first two days and then by about 35% between days 2 and 10. Anaerobic treatment was previously found suitable to eliminate the majority of paracetamol, by more than 90% (De Graaff *et al*. 2011); however, another study indicated that the removal efficiency of paracetamol in anaerobic digesters was only 11%, with a SRT of 56 days (Musson *et al.* 2010). An additional field study reported more than 80% biodegradation of paracetamol in anaerobic sludge (Narumiya *et al.* 2013). It appears from this study that paracetamol can be biodegraded by both aerobic and anaerobic microorganisms.

The biodegradation of ibuprofen was less than 20% over the 10-day period (Figure 6.2); and biodegradation started after a lag phase of about 2 days. These results are in agreement with Carballa *et al.* (2007), who showed that the

removal efficiencies of ibuprofen varied between 40% and 60%, for various initial concentrations (4-400 µg/L) under anaerobic conditions during up to 30 days incubation (or SRT). Also, Musson *et al*. (2010) showed overall reduction of ibuprofen of 28% under anaerobic conditions during 56 days experimentation for an initial concentration of 66 mg/L.

The biodegradation characteristics of naproxen under anaerobic conditions in this study were similar to those of ibuprofen. The observed average removal efficiency of naproxen after 10 days was less than 25%. Recently, Alvarino *et al*. (2014) also reported a low removal efficiency of naproxen (< 15%) in an anaerobic digestion system. In contrast, Campbell (2013) reported a high removal efficiency of naproxen (up to 100%) under anaerobic digestion over a period of 60 days (with initial concentrations of 25 and 50 mg/L). Therefore, it is possible that the low removal efficiency of naproxen and ibuprofen under anaerobic conditions observed in this study may be due to the relatively short SRTs. A longer SRT will result in more biomass that will enhance the biodegradation rates and increase the methanogenic activity rates (Alvarino *et al.* 2014).

6.3.3 Discussion of the biodegradation kinetics of selected compounds during biological treatment

The observed biodegradation behaviours of the selected pharmaceutical compounds under aerobic and anaerobic conditions are shown in Table 6.1. Although different batch durations and concentrations of biomass were used for the both studies, the main aim of this work was to provide an examination into

the behaviour of the selected pharmaceuticals under different biological conditions and to compare the relative fate of the compounds under similar biological conditions.

Table 6.1: Biodegradation rates of selected pharmaceuticals under aerobic and anaerobic conditions over 10 days.

Compound	**Average rate of biodegradation**	
	Aerobic conditions	Anaerobic conditions
Paracetamol	80%	80%
Ibuprofen	18%	20%
Naproxen	18%	25%
Sulfamethoxazole	3%	98%

It is clear that sulfamethoxazole behaved completely differently under anaerobic versus aerobic conditions; while it was nearly completely removed under anaerobic conditions, poor biodegradation of the compound was observed under aerobic conditions, over 10 days. Previous studies have also shown efficient removal rates achieved by anaerobic digestion (Mohring *et al*. 2009; Larcher and Yargeau 2011). Antibiotics, especially fluoroquinolones and macrolides, have been shown to be less biodegradable in activated sludge plants (Baquero *et al.* 2008). However, high levels of removal under anaerobic conditions were observed for sulfamethoxazole and roxithromycin (Carballa *et al.* 2007), and oxytetracycline and chlortetracycline (Álvarez *et al.* 2010). Although, sulfamethoxazole was the only antibiotic assessed in this study, other antibiotics might behave similarly. Therefore, anaerobic conditions should be suitable to remove some these compounds. Providing anaerobic treatment as

part of wastewater treatment system may offer a cheaper alternative than installing advanced technologies (with or without activated sludge systems) especially in tropical climates, where high ambient temperature is available year round.

Paracetamol appeared to be highly biodegradable under both aerobic and anaerobic conditions, with an average of 80% removal over 10 days (Table 6.1). In support of this, the results of the batch tests corroborate those from the effluent analysis of WWTPs in this study (Chapter 5), where the paracetamol present in the various WWTP influents from both the UK and Saudi Arabia was completely removed in the effluents. These findings suggest that, in general, paracetamol is a highly biodegradable compound under both aerobic and anaerobic biological treatment conditions. Consequently, if paracetamol was found to be present in the environment, it is expected that its concentrations would be relatively minimal and easily biodegradable by the microorganisms present in the surface waters.

The biodegradation of naproxen and ibuprofen under both aerobic and anaerobic conditions were relatively similar (moderate). The similarities between naproxen and ibuprofen biodegradation profiles may be due to the fact that the microorganisms may require longer acclimation periods for their biodegradation.

6.4 Conclusions

The biodegradation of selected compounds in aerobic and anaerobic batch tests revealed different biodegradability potentials. Sulfamethoxazole was

completely biodegraded under anaerobic conditions, but poorly biodegraded under aerobic conditions. Incomplete and slow biodegradation of naproxen and ibuprofen under both aerobic and anaerobic conditions was probably due to the need for relatively longer microbial adaptation periods. Conversely, paracetamol concentrations were highly biodegradable under both aerobic and anaerobic conditions.

Overall, on the basis of the biodegradation rates, the compounds can be divided into two groups. The first group (paracetamol) comprises pharmaceutical compounds that can be rapidly biodegraded under various environmental conditions. The second group (sulfamethoxazole, ibuprofen and naproxen) consists of those compounds that can be biodegraded, but only under certain conditions (e.g. longer SRT, HRT) or types of microorganisms. Where higher removal efficiencies of the pharmaceutical compounds were found, these could be explained by the different biomass and adaptation of the bacteria in the sludge over time. Therefore, the observations from the batch tests lead to the conclusion that combining anaerobic and aerobic biological treatment systems with longer SRTs could result in an overall increase in the removal efficiencies of pharmaceutical compounds.

CHAPTER 7

Proposed pharmaceutical wastewater treatment for point sources

7.1 Introduction

In most cases, hospital wastewaters (HWWs) contain very high concentrations of pharmaceutical compounds and healthcare products. These compounds, along with other chemicals, are reportedly detected at higher concentrations in HWWs than in municipal wastewaters (as shown in Chapter 5). Separate treatment of HWW onsite can be designed to target the removal of specific pollutants at the point source. Removal efficiencies can thus be increased, however the costs of on-site treatment are significant. In some cases the installation of additional equipment will be required, and this will require operation and maintenance. This imposes associated costs, and may include a large energy requirement (Chapter 2; section 2.7).

7.2 On-site treatment of hospital wastewater

Hospital wastewaters have been shown to be a significant point source of pollutants contributing to the public sewer (Chapters 2 & 5). Consequently, the separation and treatment of concentrated wastewater at the point source (e.g. hospitals) represents an interesting option, as an alternative to centralised treatment. Such treatment would occur before dilution with other wastewater in the public treatment system so is likely to be more efficient.

7.3 Hospital wastewater separation at source

Water is essential in all areas of hospitals and nursing homes. As reported in Chapter 2, the laundry, kitchen, air conditioning, heating and other sanitary facilities produce general wastewater that is not contaminated with pharmaceutical compounds. In contrast, other units, such as laboratories, patients' wards, intensive care and other medical service units, produce hazardous wastewater. Within hospital sewers, both hazardous and unhazardous wastewaters are mixed. However, because the units that generate hazardous wastewater are known, the non-hazardous wastewater units (laundry, kitchen etc.) could be directly connected to urban sewerage systems, whilst the wastewater stream containing hazardous pollutants could undergo onsite treatment. In this arrangement, better control and efficient treatment of pharmaceutical compounds, would be possible. Pharmaceutical compounds of concern would be present at higher concentrations in the wastewater stream directed to the onsite treatment system as they would not be diluted with general, non-hazardous wastewater.

7.4 Proposed treatment processes for pharmaceutical compounds

Worldwide, hospital and municipal wastewater treatment plants (WWTPs) commonly employ aerobic activated sludge processes. As shown in Chapters 5 and 6, the pharmaceutical compounds investigated in this research were affected by treatment processes under both aerobic and anaerobic conditions. In a conventional activated sludge process, the biodegradation rates of pharmaceutical compounds appeared higher under certain operational

conditions, including long SRTs and HRTs, lower levels of dilution and higher ambient temperatures. Therefore, on-site treatment and upgrading an activated sludge process to optimize the conditions could significantly increase the removal efficiencies of pharmaceutical compounds. Due to the different climatic conditions, which affect the ambient temperature, different options are proposed for the point source treatment of pharmaceutical compounds under tropical and temperate climates.

7.4.1 Tropical climate

Many of the world's regions are subject to warm climatic conditions particularly developing countries (Baruselli *et al.* 2004). A warm climate is favourable for biological treatment with bacteria, because the elevated temperatures play an important role in bacterial growth and activity during treatment (Risk and Baath 2011; Nydahl *et al.* 2013; Yang *et al.* 2014). Based on the results of this research, the integration of an anaerobic and aerobic biological treatment stage as shown in Figure 7.1 is proposed, along with longer SRTs and HRTs that will enhance the overall efficiency of biodegradation of pharmaceutical compounds.

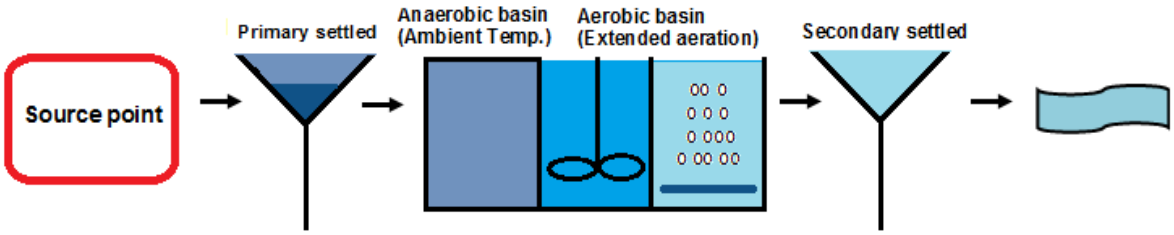

Fig. 7.1: The proposed treatment of pharmaceutical compounds under elevated temperatures (in tropical climates).

With the digestion processes maintained at ambient temperatures, anaerobic digestion is proposed as a pre-treatment (at an ambient temperature) and the aerobic treatment conditions would comprise a post-treatment, with optimised SRT and HRT. The anaerobic conditions would be important for the reduction of pharmaceuticals, especially antibiotics, and the aerobic conditions would reduce other pharmaceuticals and remaining organic pollutants. For existing activated sludge plants, upgrading to ensure longer HRTs (and also ensuring that the SRT is between 10-15 days), and using complete nitrification as an indicator is recommended. The combination of aerobic and anaerobic processes would enhance the overall efficiency of biodegradation of the pharmaceutical compounds. This process would have the additional advantage of increasing the removal efficiency of ammonia and would be more cost-effective than adding advanced treatment process units. Anaerobic–aerobic treatment has numerous advantages, such as low energy consumption, low chemical consumption, low sludge production, less equipment required and operational simplicity (Chan *et al.* 2009). In addition, for high strength wastewater, the anaerobic zone could produce methane gas and this biogas could be captured to produce renewable energy.

7.4.2 Temperate climate

The treatment of pharmaceutical compounds in wastewater under low ambient temperature conditions may only lead to limited biodegradation of the pollutants under anaerobic digestion (Bowen *et al.* 2014; Zeman *et al.* 2014). Therefore, in temperate climates it is likely that the anaerobic treatment process would need

to be heated to above 30°C to optimise biodegradation rates. However, heating of the anaerobic zone during treatment could be costly. Therefore, as an alternative, advanced treatment processes could be used and are proposed for use in temperate climates as shown in Figure 7.2.

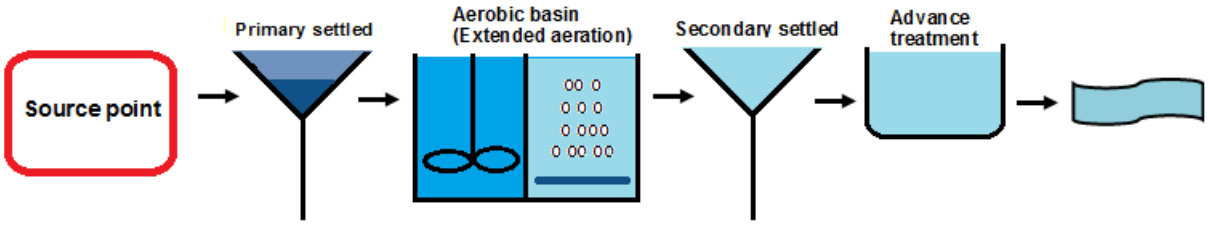

Fig. 7.2: The proposed treatment of pharmaceutical compounds under low temperatures (in temperate climates).

From the results obtained in this study of the performance of some WWTPs in the UK, upgrading activated sludge processes to a total SRT of 15 days or more with a longer HRT (>15 hours) is recommended. When the SRT in the aeration tank is sufficiently long, the removal of pharmaceutical compounds during the aerobic treatment process may be enhanced. An extended aeration system is likely to ensure the effective adaptation of microbial communities to the micro-organic pollutants in the aeration tank. Alternatively, extended aeration and longer SRTs can be obtained by the use of membrane bioreactors (MBRs). MBRs also have the advantage of eliminating the use of a secondary sedimentation tank for biomass-treated effluent separation (Akunna and Bartie 2014), thereby making it ideal for use where space is limited.

Pharmaceutical compounds, such as carbamazepine, that are resistant or recalcitrant to microbial degradation under sub-optimal temperature conditions (i.e., in a temperate climate), can be treated with an additional advanced

treatment such as activated carbon. Activated carbon is recommended to reduce the emissions of these compounds into the environment. Although activated carbon can be costly, the amount used depends on the concentrations of the compounds released from the secondary (activated sludge) treatment. It is expected that the amount of activated carbon used will be affordable if used as a post-treatment step, where an extended aeration system, as proposed in Figure 7.2, is optimised to remove most of the readily and non-readily biodegradable compounds prior to post-treatment.

CHAPTER 8

General conclusions

The aim of this thesis was to contribute to the knowledge concerning the occurrence and behaviour of pharmaceutical compounds during municipal and hospital wastewater treatment under different environmental conditions. The main conclusions and results obtained from this research are summarised below.

8.1 The occurrence of pharmaceutical compounds in municipal and hospital wastewaters

Samples from the influent and effluent of conventional municipal and hospital wastewater treatment plants in the UK and Saudi Arabia were analysed to investigate the occurrence and elimination of selected pharmaceutical compounds. The highest concentrations of pharmaceutical compounds measured in the influents of municipal WWTPs (> 1 µg/L) were of paracetamol, naproxen and bezafibrate, followed by carbamazepine, atenolol, lidocaine, sulfamethoxazole and NACS, the concentrations of which were less than 1 µg/L. In the hospital WWTPs, the highest concentrations of pharmaceutical compounds measured (> 10 µg/L) were of paracetamol and caffeine, followed by ciprofloxacin and NACS (1–6 µg/L), and finally bezafibrate, carbamazepine, atenolol, lidocaine, clarithromycin and sulfamethoxazole (< 1 µg/L). In general, the hospital wastewater contained relatively higher levels of the pharmaceutical compounds measured than the municipal wastewater. Various antibiotics were

frequently detected in the hospital wastewater, while only one type of antibiotic was detected in the municipal wastewater.

8.2 The effect of operational parameters on the removal efficiency of pharmaceutical compounds in wastewater treatment plants

8.2.1 Solids retention time and hydraulic retention time

Higher removal efficiencies of paracetamol, naproxen, bezafibrate and atenolol were achieved in activated sludge processes operating with longer hydraulic retention times (HRTs > 15 h) and solids retention times (> 10 days, as indicated by the occurrence of effective nitrification) than those with shorter HRTs and SRTs in the UK WWTPs. A longer SRT seemed to promote the adaptation of different kinds of microorganisms, including slow growing species that could have a greater capacity for removing the compounds of interest.

8.2.2 Ambient temperature

In terms of the pharmaceutical compounds investigated, greater removal efficiencies were achieved for atenolol, carbamazepine, lidocaine and antibiotics in an activated sludge plant in a tropical climate. These compounds are normally found to be persistent at conventional WWTPs in temperate climates. The higher ambient temperatures (> 26°C), which are present almost year-round in the tropical climate, could bring about higher levels of microbial activity and ensure increased biodegradation kinetics of organic pollutants in the wastewater.

8.2.3 Dilution factor

Where hospital wastewater is co-treated with municipal wastewater, as in the UK, high volumes of municipal wastewater may dilute the overall concentrations of the pharmaceutical compounds in the combined wastewater. Similarly, rainfall reaching the combined system can also contribute to dilution of the concentrations of pharmaceuticals present in the wastewater. In this study, it was found that the removal efficiencies of pharmaceutical compounds were considerably greater in the dry tropical conditions in Saudi Arabia than in wet weather conditions in the UK, even though the concentrations of these compounds in the UK wastewater were lower. This may be due to the effect of dilution, although more research is needed to draw explicit conclusions. Even though poor removal rates were achieved during wet weather conditions, the effects of dilution would have also reduced the possible environmental hazards posed by the compounds to aquatic organisms.

8.3 The effect of anaerobic and aerobic biodegradation

In order to compare biodegradation rates under aerobic and anaerobic conditions, laboratory biodegradation experiments were conducted. Aerobic and anaerobic digestion processes were operated for extended periods in the laboratory and the biodegradation rates of four pharmaceutical compounds were measured. The biodegradation rates varied significantly ranging from poor to high depending on the retention period considered. High levels of paracetamol were removed (> 90%) during both the aerobic and anaerobic conditions. Ibuprofen and naproxen elimination rates were moderate under both

aerobic and anaerobic conditions, while the elimination of sulfamethoxazole was poor under aerobic and high under anaerobic conditions. The most relevant impact on the removal of the compounds was the SRT and the type of microorganisms used (i.e. aerobic vs. anaerobic). A longer SRT enhanced the adaptation of microorganisms and increased the microbial activity, which improved the removal efficiencies of the pharmaceutical compounds. The results thus confirmed observations from the treatment plant studies.

8.4 Proposed treatment processes for pharmaceutical compounds

The proposed treatment process recommends the separation of hospital wastewater. Under tropical conditions, combining aerobic and anaerobic treatment with longer SRTs (10-15 days) and HRTs (> 10 h) is recommended to enhance the overall biodegradation efficiency of most readily and non-readily biodegradable pharmaceutical compounds in municipal and hospital wastewaters. Advanced processes, can be added as post-treatment to remove compounds that are resistant or recalcitrant to microbial degradation in temperate conditions.

8.5 Recommendations for future research

The knowledge about the fate and behaviour of pharmaceutical compounds in wastewater gathered from this work confirms that the degree of biodegradation of pharmaceutical compounds in conventional activated sludge systems depends on factors, such as the type of process (aerobic or anaerobic), SRT

and temperature. Based on these results and the conclusions of this work, the following recommendations for further research can be made:

- In this research, parent compounds and their metabolites were detected in the collected samples. However, only the parent compounds were analysed, except for the metabolites of sulfamethoxazole (NACS). More research is therefore needed in order to understand the fate of the produced metabolites and their biodegradability.
- It is necessary to investigate the impacts of long-term exposure of microorganisms to pharmaceutical compounds in water systems, along with the wider environmental risks.
- The analysis of pharmaceutical compounds at low concentrations currently presents a challenge. More research is recommended to improve the accuracy and ease of analysis of pharmaceutical compounds.

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APPENDICES

UK Sampling data

BOD_5 (mg/L)

No.	Date	Plant	Influent	Effluent
1	18/09/12	Letham	34.53	4.37
2	19/09/12		38.41	4.68
3	26/09/12		22.07	5.36
4	03/10/12		52.46	12.78
5	05/10/12		--	6.85
6	10/10/12		74.12	8.03
7	12/10/12		--	10.98
8	17/10/12		32.21	6.59
9	18/10/12		--	6.25
10	23/10/12		--	6.52
11	24/10/12		--	7.67
12	30/10/12		--	6.04
13	01/11/12		--	5.93
14	06/11/12		43.00	8.82
15	09/11/12		61.87	7.52

No.	Date	Plant	Influent	Effluent
1	18/09/12	Cupar	156.26	2.16
2	19/09/12		181.96	0.28
3	26/09/12		130.24	2.52
4	03/10/12		201.80	9.17
5	05/10/12		--	8.67
6	10/10/12		225.92	7.45
7	12/10/12		--	13.87
8	17/10/12		144.30	6.49
9	18/10/12		--	4.76
10	23/10/12		--	3.74
11	24/10/12		--	5.75
12	30/10/12		--	3.59
13	01/11/12		--	5.44
14	06/11/12		229.60	4.13
15	09/11/12		256.76	3.93

No.		Plant	Influent	Effluent
1	18/09/12	Guardbridge	33.61	5.83
2	19/09/12		72.51	3.03
3	26/09/12		42.42	9.13
4	03/10/12		62.14	7.76
5	05/10/12		--	7.73
6	10/10/12		105.56	7.16
7	12/10/12		--	9.74
8	17/10/12		56.76	4.98
9	18/10/12		--	8.05
10	23/10/12		--	3.57
11	24/10/12		--	5.07
12	30/10/12		--	1.86
13	01/11/12		--	5.93
14	06/11/12		58.40	4.69
15	09/11/12		87.75	2.89

No.	Date	Plant	Influent	Effluent
1	03/10/12	Hatton	25.95	1.55
2	10/10/12		64.10	0.42
3	12/10/12		89.71	5.17
4	15/10/12		48.40	7.52
5	18/10/12		--	4.36
6	30/10/12		--	3.66
7	01/11/12		--	5.81
8	06/11/12		34.96	4.10
9	09/11/12		61.84	3.52

COD (mg/L)

No.	Date	Plant	Influent	Effluent
1	18/09/12	Letham	175	33.4
2	19/09/12		132	29.6
3	26/09/12		70.2	31.4
4	03/10/12		101	32.7
5	05/10/12		--	28.1
6	10/10/12		158	34.1
7	12/10/12		--	29.7
8	17/10/12		61.4	21.8
9	18/10/12		--	23.5
10	23/10/12		--	30.7
11	24/10/12		--	20.2
12	30/10/12		109	34.3
13	01/11/12		--	21.2
14	06/11/12		104	37.2
15	09/11/12		145	39.8

No.	Date	Plant	Influent	Effluent
1	18/09/12	Cupar	470	22.4
2	19/09/12		491	20.5
3	26/09/12		354	26.7
4	03/10/12		344	24.1
5	05/10/12		--	35.9
6	10/10/12		464	28.0
7	12/10/12		--	26.9
8	17/10/12		233	22.1
9	18/10/12		--	21.8
10	23/10/12		--	19.1
11	24/10/12		--	17.0
12	30/10/12		317	17.0
13	01/11/12		--	18.3
14	06/11/12		487	15.9
15	09/11/12		607	22.7

No.	Date	Plant	Influent	Effluent
1	18/09/12	Guardbridge	136	13.6
2	19/09/12		182	16.5
3	26/09/12		130	39.4
4	03/10/12		109	19.1
5	05/10/12		--	18.3
6	10/10/12		232	18.3
7	12/10/12		--	22.9
8	17/10/12		143	18.2
9	18/10/12		--	24.0
10	23/10/12		--	13.5
11	24/10/12		--	17.5
12	30/10/12		91.6	16.0
13	01/11/12		--	18.6
14	06/11/12		123	15.2
15	09/11/12		199	20.8

No.	Date	Plant	Influent	Effluent
1	03/10/12	Hatton	78.5	22.0
2	10/10/12		177	20.7
3	12/10/12		119	18.2
4	15/10/12		68.6	23.6
5	18/10/12		--	16.8
6	30/10/12		157	19.8
7	01/11/12		81.7	24.4
8	06/11/12		81.7	28.6
9	09/11/12		142	26.3

TOC (mg/L)

No.	Date	Plant	Influent	Effluent
1	18/09/12	Letham	45.8	9.73
2	19/09/12		59.2	11.7
3	26/09/12		30.1	10.8
4	03/10/12		38.7	6.39
5	05/10/12		--	10.1
6	10/10/12		65.3	11.4
7	12/10/12		--	8.13
8	17/10/12		29.6	7.33
9	18/10/12		--	6.88
10	23/10/12		--	12.0
11	24/10/12		--	4.81
12	30/10/12		43.9	15.3
13	01/11/12		--	4.65
14	06/11/12		44.4	12.0
15	09/11/12		37.4	13.8

No.	Date	Plant	Influent	Effluent
1	18/09/12	Cupar	137	7.19
2	19/09/12		225	9.46
3	26/09/12		117	2.97
4	03/10/12		110	2.58
5	05/10/12		--	6.48
6	10/10/12		145	9.85
7	12/10/12		--	7.31
8	17/10/12		61.9	5.08
9	18/10/12		--	7.52
10	23/10/12		--	6.82
11	24/10/12		--	8.34
12	30/10/12		110	6.96
13	01/11/12		--	7.65
14	06/11/12		126	5.82
15	09/11/12		181	8.12

No.	Date	Plant	Influent	Effluent
1	18/09/12	Guardbridge	41.0	9.48
2	19/09/12		71.4	9.00
3	26/09/12		42.5	7.09
4	03/10/12		53.5	3.19
5	05/10/12		--	3.99
6	10/10/12		105	2.89
7	12/10/12		--	4.90
8	17/10/12		56.6	2.96
9	18/10/12		--	6.61
10	23/10/12		--	3.70
11	24/10/12		--	7.50
12	30/10/12		28.8	5.84
13	01/11/12		--	4.12
14	06/11/12		35.3	5.69
15	09/11/12		35.1	6.45

No.	Date	Plant	Influent	Effluent
1	03/10/12	Hatton	37.3	9.07
2	10/10/12		57.4	8.83
3	12/10/12		44.4	10.5
4	15/10/12		50.8	7.59
5	18/10/12		--	6.19
6	30/10/12		91.1	5.90
7	01/11/12		--	4.14
8	06/11/12		43.1	10.2
9	09/11/12		53.8	5.47

NH_4 (mg/L)

No.	Date	Plant	Influent	Effluent
1	18/09/12	Letham	15.2	1.56
2	19/09/12		16.9	1.23
3	26/09/12		3.02	0.32
4	03/10/12		7.96	0.55
5	05/10/12		--	0.00
6	10/10/12		16.4	0.99
7	12/10/12		--	0.00
8	17/10/12		3.60	0.00
9	18/10/12		--	0.00
10	23/10/12		--	0.24
11	24/10/12			0.20
12	30/10/12		5.35	1.72
13	01/11/12		--	0.72
14	06/11/12		13.7	1.69
15	09/11/12		19.7	1.38

No.	Date	Plant	Influent	Effluent
1	18/09/12	Cupar	22.0	0.99
2	19/09/12		47.5	1.60
3	26/09/12		11.8	0.13
4	03/10/12		17.7	0.49
5	05/10/12		--	0.00
6	10/10/12		34.6	0.00
7	12/10/12		--	0.00
8	17/10/12		18.6	0.00
9	18/10/12		--	0.00
10	23/10/12		--	0.05
11	24/10/12		--	0.04
12	30/10/12		20.4	0.05
13	01/11/12		--	0.46
14	06/11/12		21.0	0.075
15	09/11/12		25.1	0.134

No.	Date	Plant	Influent	Effluent
1	18/09/12	Guardbridge	18.9	0.13
2	19/09/12		31.4	0.00
3	26/09/12		11.5	0.00
4	03/10/12		13.6	0.05
5	05/10/12		--	0.00
6	10/10/12		39.1	0.00
7	12/10/12		--	0.00
8	17/10/12		15.8	0.02
9	18/10/12		--	0.00
10	23/10/12		--	0.06
11	24/10/12		--	0.03
12	30/10/12		13.6	0.05
13	01/11/12		--	0.21
14	06/11/12		14.6	0.095
15	09/11/12		31.0	0.569

No.	Date	Plant	Influent	Effluent
1	03/10/12	Hatton	22.1	23.9
2	10/10/12		32.5	30.2
3	12/10/12		11.2	12.7
4	15/10/12		15.6	18.1
5	18/10/12		--	8.40
6	30/10/12		24.6	17.6
7	01/11/12		--	16.3
8	06/11/12		18.5	22.7
9	09/11/12		27.9	19.9

NO_3 (mg/L)

No.	Date	Plant	Influent	Effluent
1	18/09/12	Letham	0.00	54.7
2	19/09/12		0.00	63.5
3	26/09/12		18.7	34.5
4	03/10/12		11.5	54.1
5	05/10/12		--	47.6
6	10/10/12		14.8	72.6
7	12/10/12		--	19.7
8	17/10/12		24.9	44.2
9	18/10/12		--	36.6
10	23/10/12		--	35.5
11	24/10/12		--	44.1
12	30/10/12		15.5	79.0
13	01/11/12		--	54.7
14	06/11/12		12.8	67.2
15	09/11/12		16.1	63.2

No.	Date	Plant	Influent	Effluent
1	18/09/12	Cupar	0.00	25.3
2	19/09/12		0.00	23.3
3	26/09/12		7.84	24.4
4	03/10/12		5.36	31.7
5	05/10/12		--	28.1
6	10/10/12		5.32	40.4
7	12/10/12		--	17.7
8	17/10/12		15.6	38.3
9	18/10/12		--	32.3
10	23/10/12		--	45.5
11	24/10/12		--	42.3
12	30/10/12		0.00	42.5
13	01/11/12		--	37.5
14	06/11/12		1.45	41.7
15	09/11/12		7.28	33.7

No.	Date	Plant	Influent	Effluent
1	18/09/12	Guardbridge	0.51	66.1
2	19/09/12		0.00	51.8
3	26/09/12		12.9	47.7
4	03/10/12		17.5	74.7
5	05/10/12		--	70.9
6	10/10/12		4.46	75.4
7	12/10/12		--	25.9
8	17/10/12		20.2	52.6
9	18/10/12		--	31.2
10	23/10/12		--	67.1
11	24/10/12		--	61.5
12	30/10/12		11.9	74.8
13	01/11/12		--	62.7
14	06/11/12		7.16	75.5
15	09/11/12		7.33	71.6

No.	Date	Plant	Influent	Effluent
1	03/10/12	Hatton	1.39	0.00
2	10/10/12		0	1.94
3	12/10/12		5.16	0.00
4	15/10/13		3.52	0.00
5	18/10/12		0.00	0.00
6	30/10/12		0.00	7.68
7	01/11/12		0.00	0.00
8	06/11/12		0.00	0.00
9	09/11/12		0.00	0.00

Saudi sampling data

COD (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	IHWWTP	348	25
2	04/04/14		317	26
3	07/04/14		344	30
4	10/04/14		-	-
5	15/04/14		321	24
6	18/04/14		349	28
7	23/04/14		-	
8	28/04/14		339	26

NH_4 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	IHWWTP	16.2	0.0
2	04/04/14		13.1	0.0
3	07/04/14		27.6	0.0
4	10/04/14			
5	15/04/14		12.6	0.0
6	18/04/14		23.5	0.0
7	23/04/14		-	-
8	28/04/14		20.9	0.0

NO_2 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	IHWWTP	0.70	0.29
2	04/04/14		0.34	0.3
3	07/04/14		0.02	0.0
4	10/04/14		-	-
5	15/04/14		0.35	0.39
6	18/04/14		0.31	0.25
7	23/04/14		-	-
8	28/04/14		0.28	0.26

NO_3 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	IHWWTP	0.43	2.49
2	04/04/14		0.33	3.1
3	07/04/14		0.32	1.54
4	10/04/14		-	-
5	15/04/14		0.32	2.13
6	18/04/14		0.29	3.12
7	23/04/14		-	-
8	28/04/14		0.39	2.44

Saudi sampling data

COD (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	SHWWTP	476	74
2	04/04/14		294	58
3	07/04/14		358	66
4	10/04/14		-	-
5	15/04/14		350	56
6	18/04/14		406	69
7	23/04/14		-	-
8	28/04/14		371	58

NH_4 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	SHWWTP	14.25	0.00
2	04/04/14		32.50	0.24
3	07/04/14		23.58	0.38
4	10/04/14		-	-
5	15/04/14		18.9	0.31
6	18/04/14		26.10	0.31
7	23/04/14		-	-
8	28/04/14		19.00	0.29

NO_2 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	SHWWTP	0.39	1.01
2	04/04/14		0.36	0.55
3	07/04/14		0.10	0.07
4	10/04/14		-	-
5	15/04/14		0.41	0.73
6	18/04/14		0.31	0.63
7	23/04/14		-	-
8	28/04/14		0.26	0.69

NO_3 (mg/l)

No.	Date	Plant	Influent	Effluent
1	02/04/14	SHWWTP	0.12	0.90
2	04/04/14		0.14	0.62
3	07/04/14		0.34	0.51
4	10/04/14		-	-
5	15/04/14		0.3	0.66
6	18/04/14		0.29	0.70
7	23/04/14		-	-
8	28/04/14		0.19	0.61

Sampling for measurement of pharmaceutical compounds

No.	Date	Time	UK Plants			
			Hatton	Cupar	Guardbridge	Letham
1	03/10/12	9:00-14:00	✓	✓	✓	✓
2	05/10/12	9:00-14:00	-	✓	✓	✓
3	10/10/12	9:00-14:00	✓	✓	✓	✓
4	12/10/12	9:00-14:00	✓	✓	✓	✓
5	15/10/12	10:00	✓	-	-	-
6	17/10/12	9:00-14:00	-	✓	✓	✓
7	18/10/12	9:00-14:00	✓	✓	✓	✓
8	01/11/13	11:00	✓	-	-	-
9	06/11/12	9:00-14:00	✓	✓	✓	✓
10	09/11/12	9:00-14:00	✓	✓	✓	✓
11	3/6/2013	9:00-14:00	✓	✓	✓	✓
12	06/06/13	9:00-14:00	✓	✓	✓	✓
13	11/06/13	9:00-14:00	✓	✓	✓	✓
14	14/06/13	9:00-14:00	✓	✓	✓	✓
15	18/06/13	9:00-14:00	✓	✓	✓	✓
16	21/06/13	9:00-14:00	✓	✓	✓	✓
17	25/06/13	9:00-14:00	✓	✓	✓	✓
18	28/06/13	9:00-14:00	✓	✓	✓	✓
			Saudi Arabia plants			
			Salman hospital		Iman hospital	
19	02/04/14	11:00-14:00	✓		✓	
21	04/04/14	11:00-14:00	✓		✓	
22	07/04/14	11:00-14:00	✓		✓	
23	10/04/14	11:00-14:00	✓		✓	
24	15/04/14	11:00-14:00	✓		✓	
25	18/04/14	11:00-14:00	✓		✓	
26	23/04/14	11:00-14:00	✓		✓	
27	28/04/14	11:00-14:00	✓		✓	

UK sample analysis

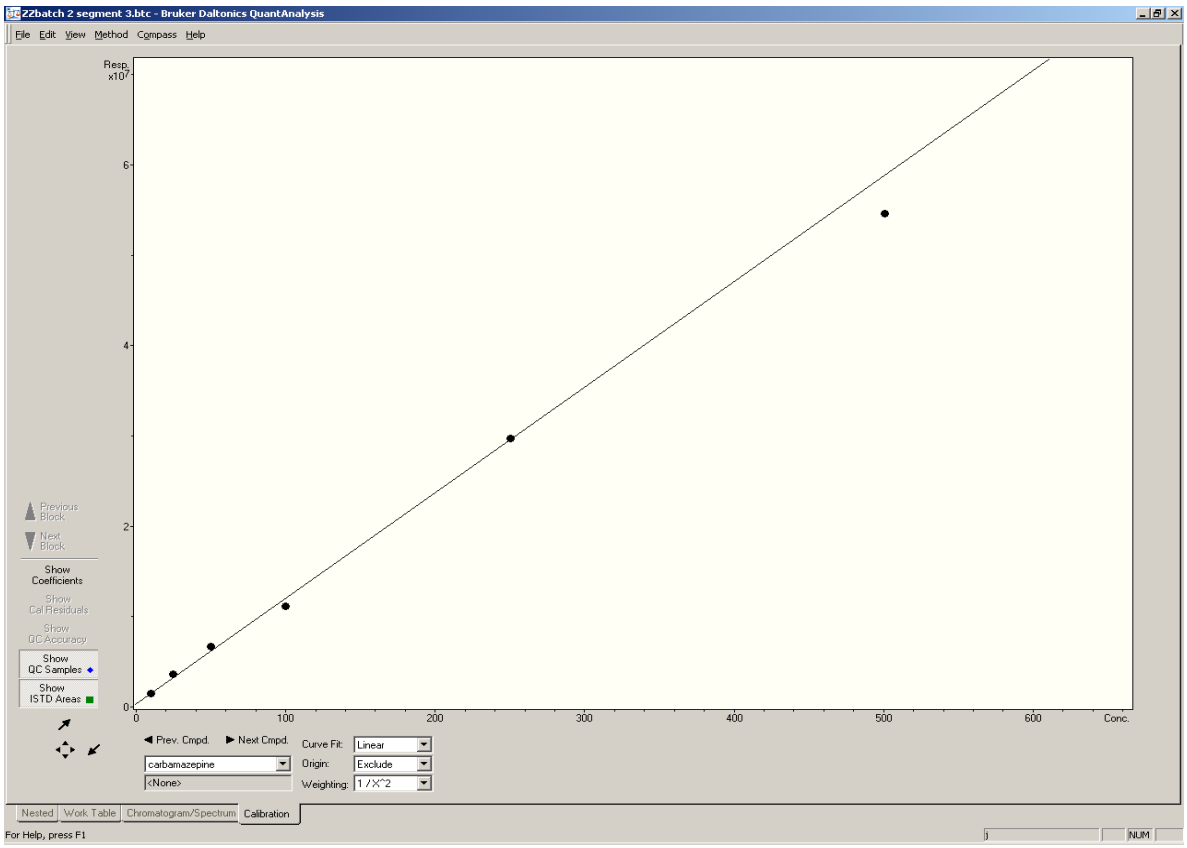
UK samples were analysed by LC-MS/MS (Agilent 1100 series LC system, auto-sampler).

HPLC conditions:

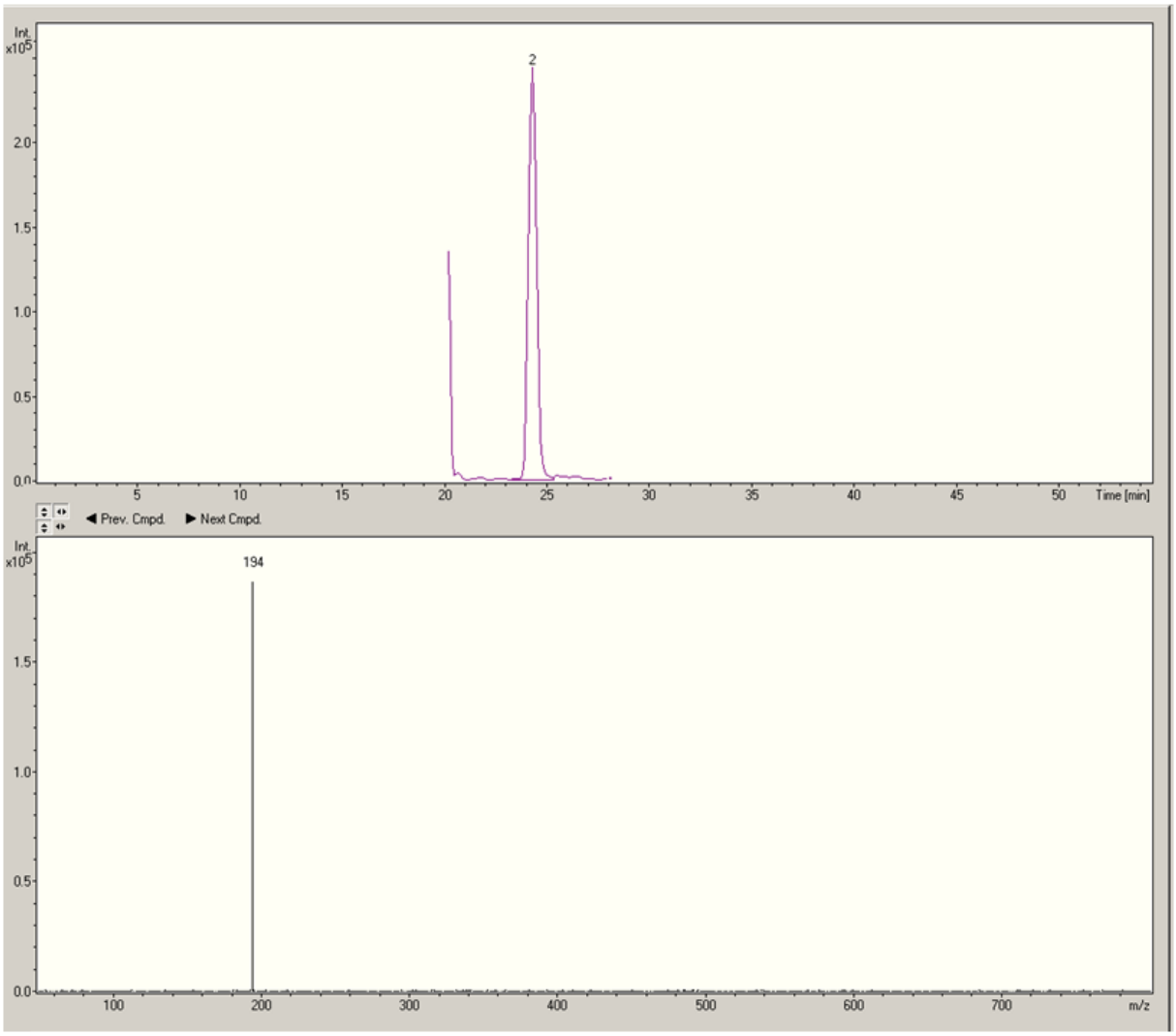
Column: Atlantis dC18 3um 2.1 x 150mm
Mobile Phase A: acetonitrile
Mobile Phase B: ammonium formate (10 mM)/formic acid/water (pH 3.5)
Time: 0-45 min

Analyte	Precursor	Product ion	Amplitude	Retention time (min)	Mode
Atenolol	267.1	190.0	0.45	10	Positive
Iopamodol	778.0	614.7	0.50	2.8	Positive
Ioprimide	791.8	773.0	0.50	2.7	Positive
Lidocaine	235.1	773.8	0.50	2.7	Positive
Ciprofloxacin	331.9	288.3	0.90	12.8	Positive
Cyclophosphamide	261.0	139.9	0.45	16.5	Positive
Sulfamethoxazole	254.0	155.9	0.40	15.8	Positive
Ifosfamide	261.0	155.9	0.40	15.8	Positive
NASC	296.0	136.0	0.60	16.6	Positive
Carbamazepine	237.0	194.0	0.60	25.6	Positive
Clarithromycin	748.4	590.3	0.30	30.8	Positive
Bezafibrate	362.1	316.0	0.45	32	Positive
Naproxen	231.0	185.0	0.50	32	Positive
Ibuprofen	204.8	160.8	0.70	34.9	Negative

Precursor and product ions showing the amplitude for fragmentation and retention time. Nebuliser Gas 40 psi, Dry Gas 7l/min and Dry Temp was 340^{o}C.



Calibration Curve for carbamazepine



Chromatogram for carbamazepine in SRM mode and the product ion scan

Saudi samples analysis

Saudi samples were analysed by LC-MS/MS (Thermo Scientific Q Exactive Quadrupole-Orbitrap Mass Spectrometer).

HPLC conditions:

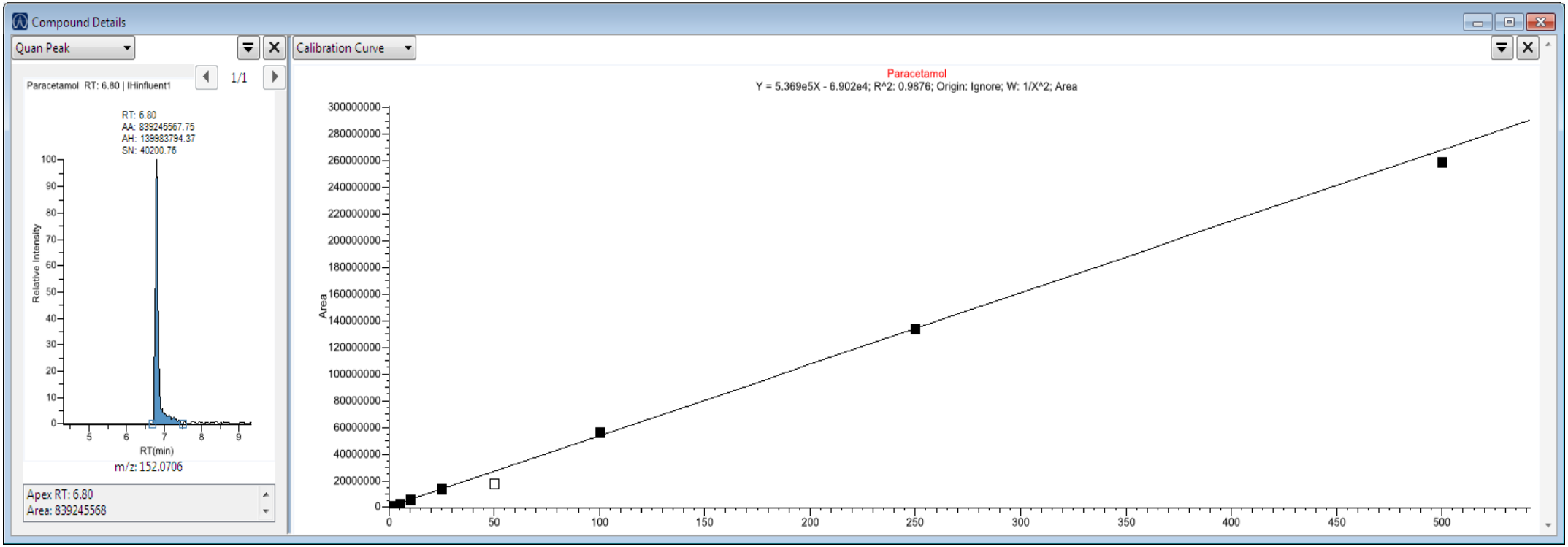
Column: Waters Xselect HSS T3. 2.1 x 150 mm (30 ± 2°C)
Mobile Phase A: acetonitrile
Mobile Phase B: ammonium formate (10 mM)/formic acid/water (pH 3.5)
Times: 0-33 min

Time (min)	A%	B%	Flow rate (ml/min)
0.00	1	99	0.2
2.00	1	99	0.2
5.00	30	70	0.2
15.00	30	70	0.2
20.00	99	1	0.2
25.00	99	1	0.2
26.00	1	99	0.2
33.00	1	99	0.2

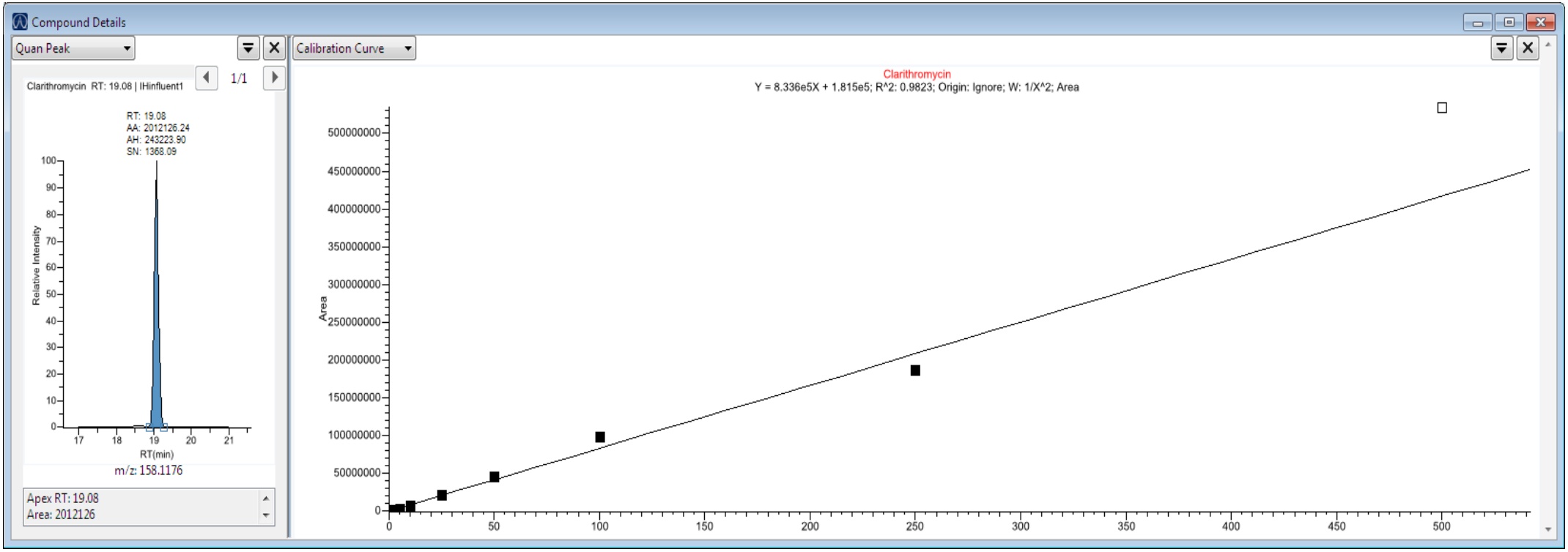
Mass spectrometry: positive ion mode.

Analyte	MS ion/transition	MS experiment
Atenolol	267.1703	full scan
Bezafibrate	362.11-316.1089	targeted MS^2
Carbamazepine	237.10-194.0964	targeted MS^2
Caffeine	195.0877	full scan
Ciprofloxacin	332.14-288.1505	targeted MS^2
Clarithromycin	748.48-158.1176	targeted MS^2
Cyclophosphamide	261.03-140.0029	targeted MS^2
Erythromycin	734.47-158.1176	targeted MS^2
Lidocaine	235.1805	full scan
NACS	296.07-134.0602	targeted MS^2
Paracetamol	152.0706	full scan
Sulphamethoxazole	254.06-108.0445	targeted MS^2

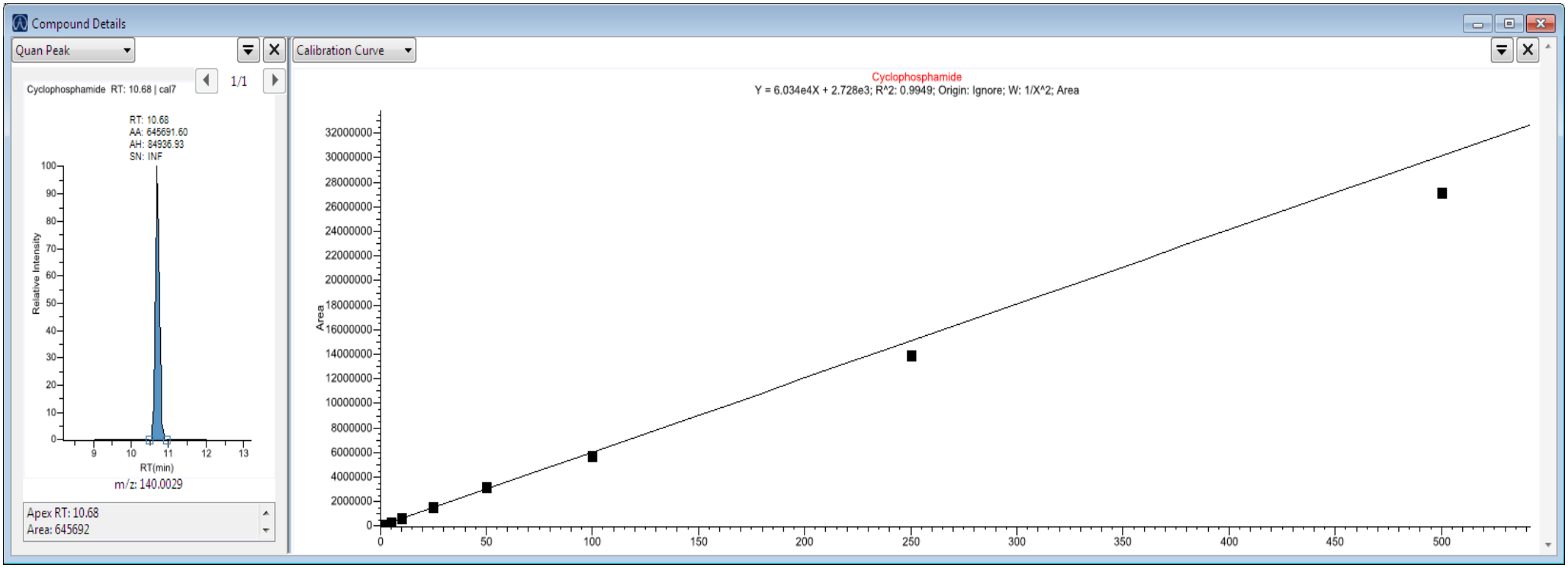
Calibration Curve and chromatogram for paracetamol



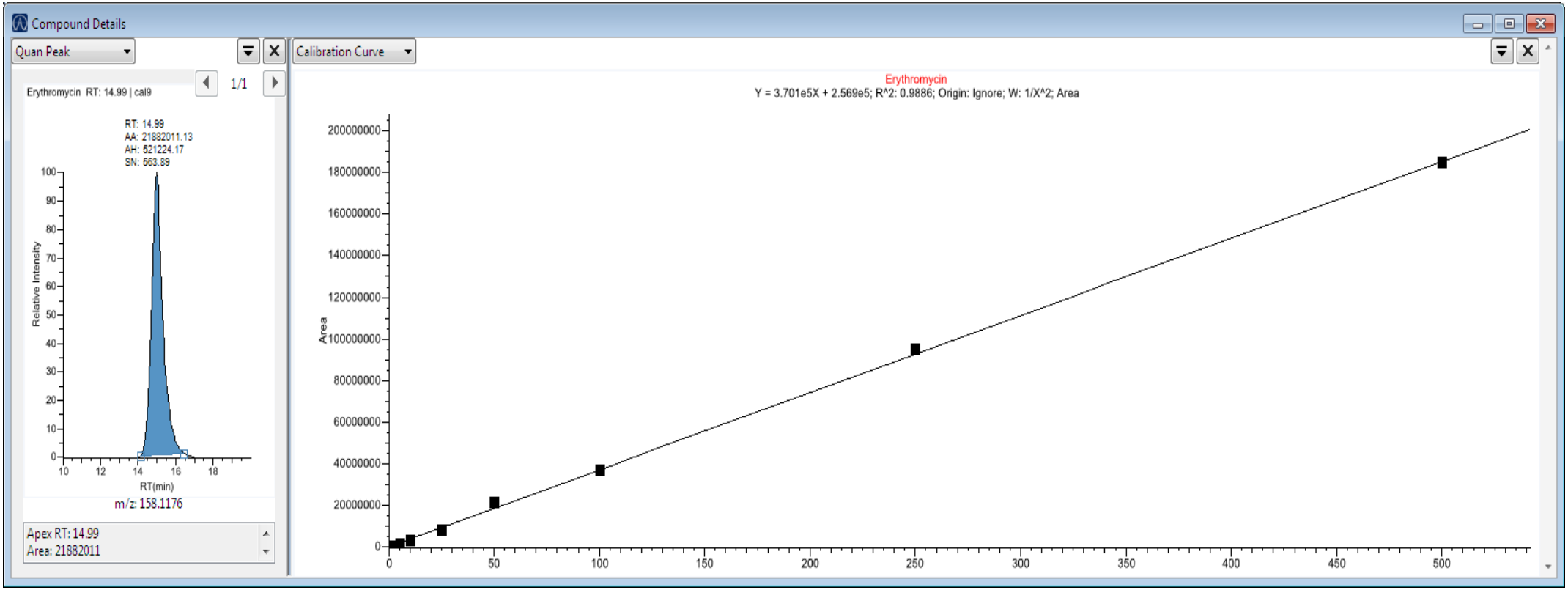
Calibration Curve and chromatogram for clarithromycin



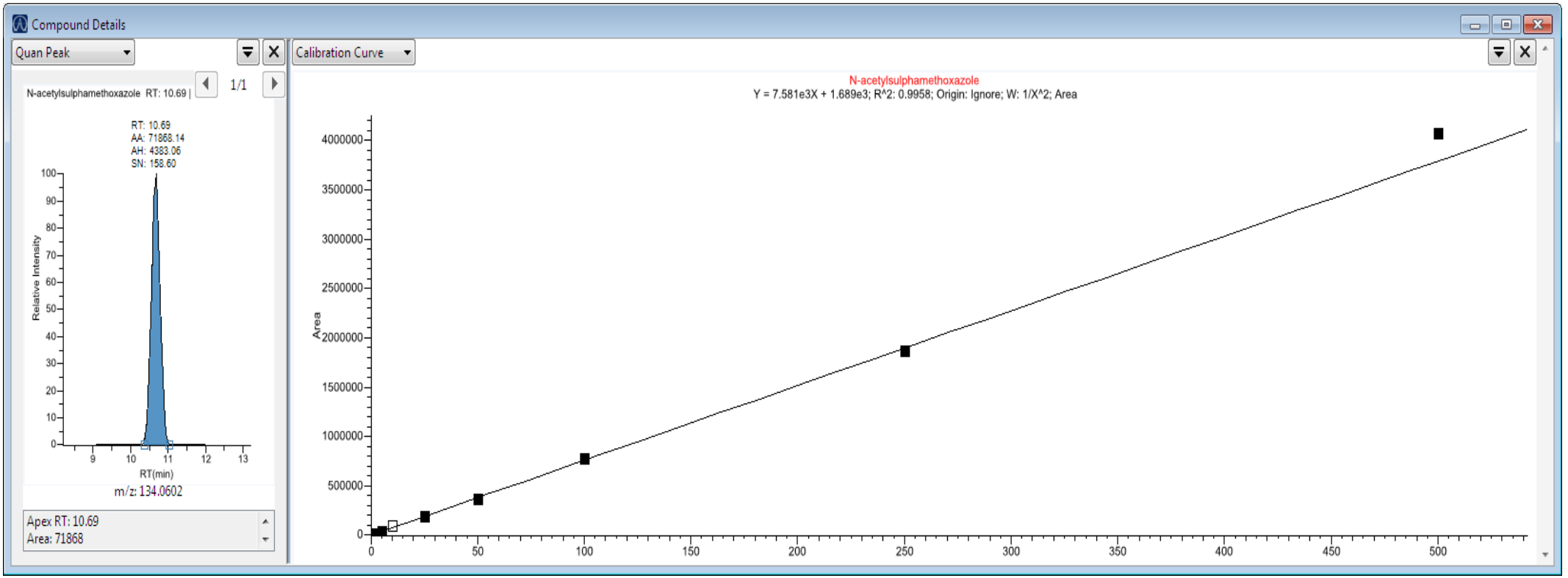
Calibration curve and chromatogram for cyclophosphamide



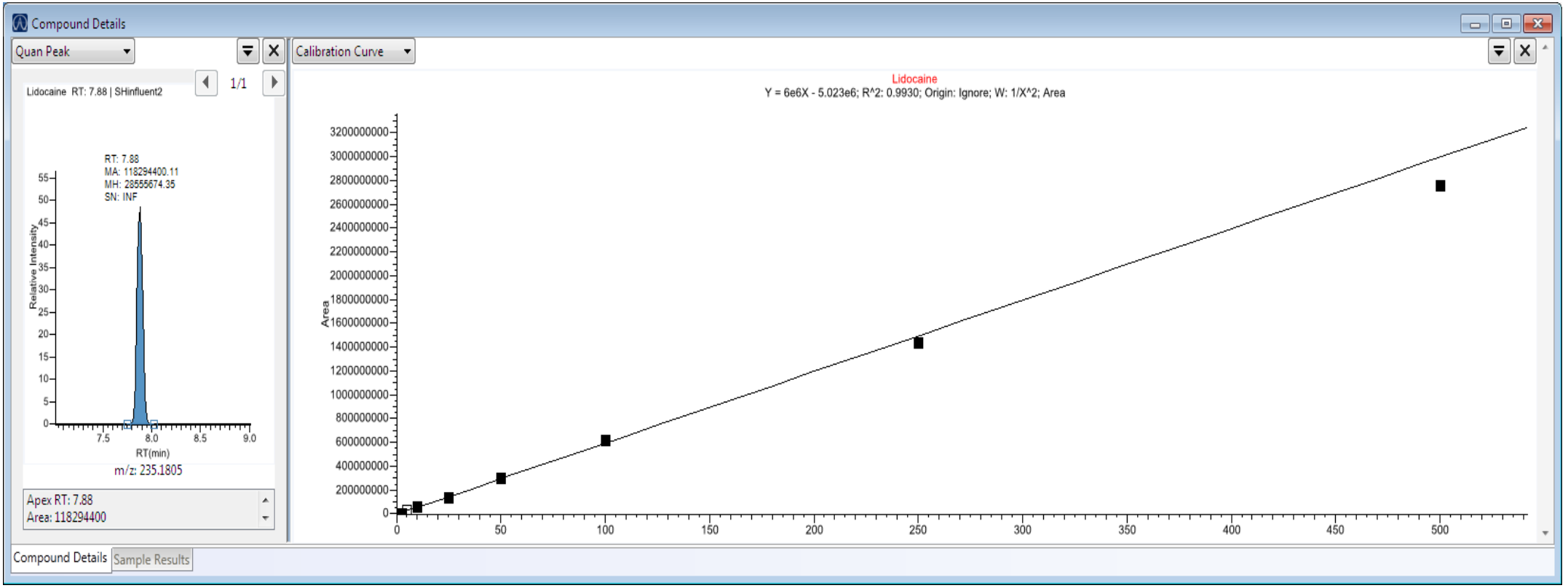
Calibration curve and chromatogram for erythromycin



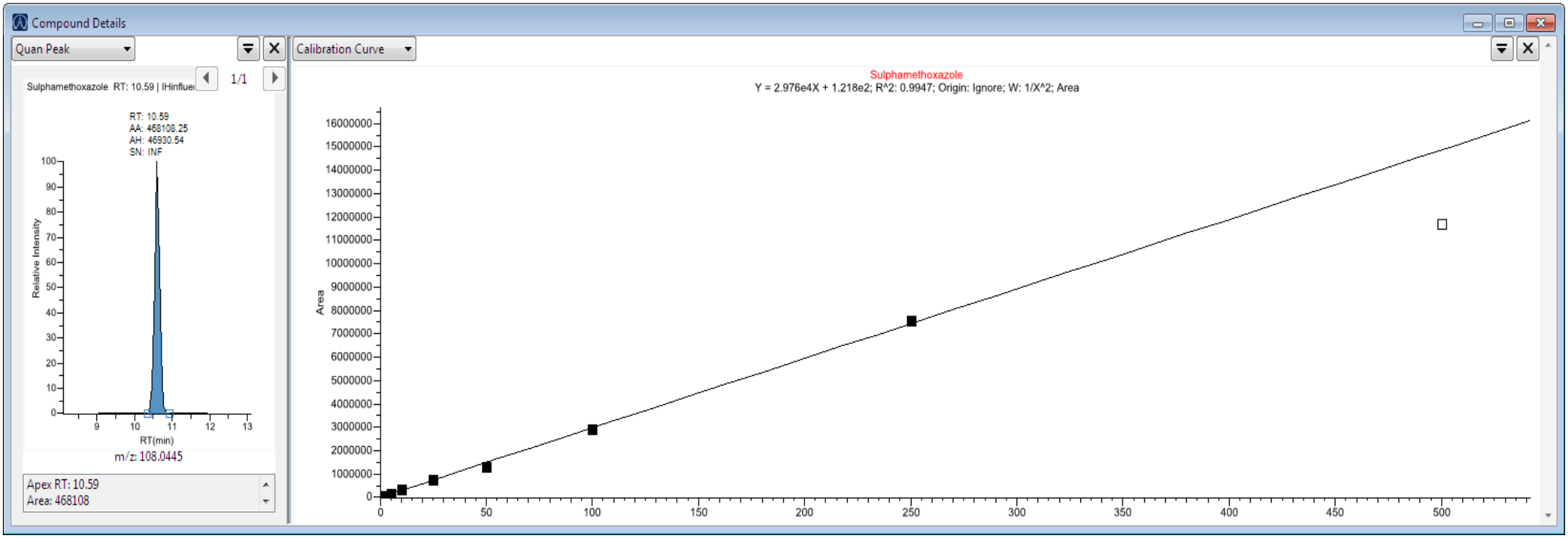
Calibration curve and chromatogram for NACS



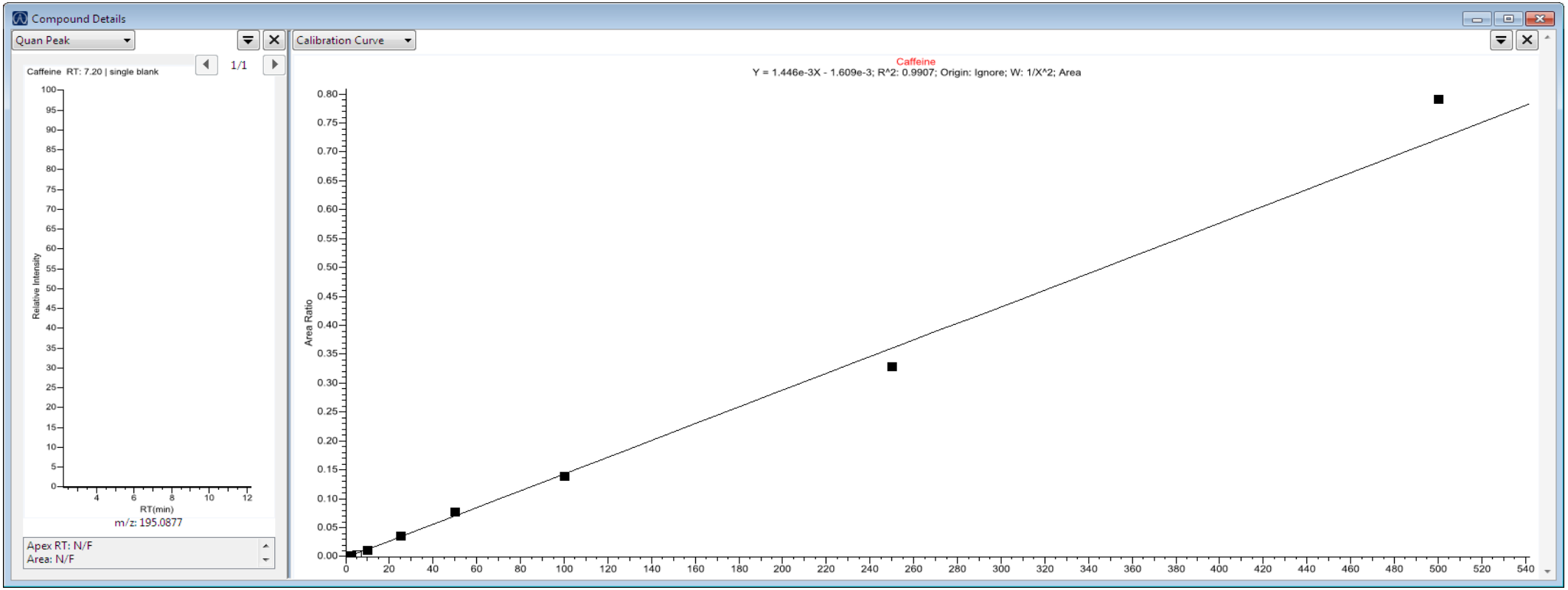
Calibration curve and chromatogram for lidocaine



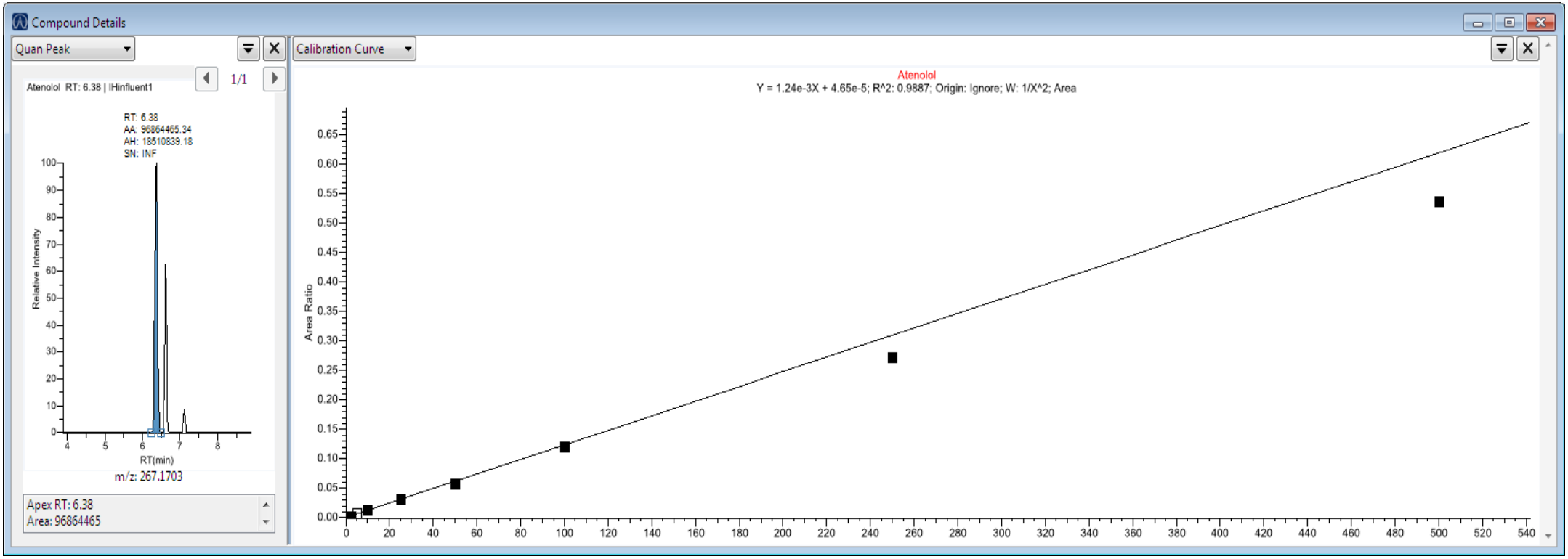
Calibration curve and chromatogram for Sulfamethoxazole



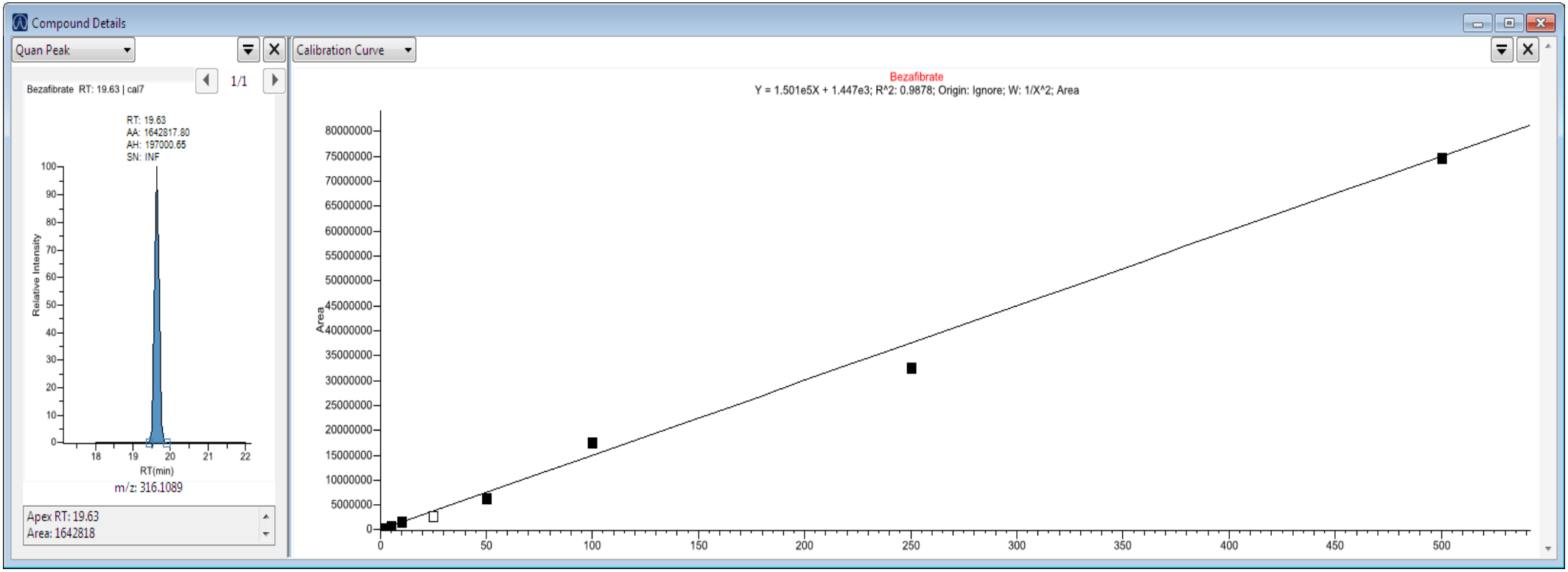
Calibration curve and chromatogram for caffeine



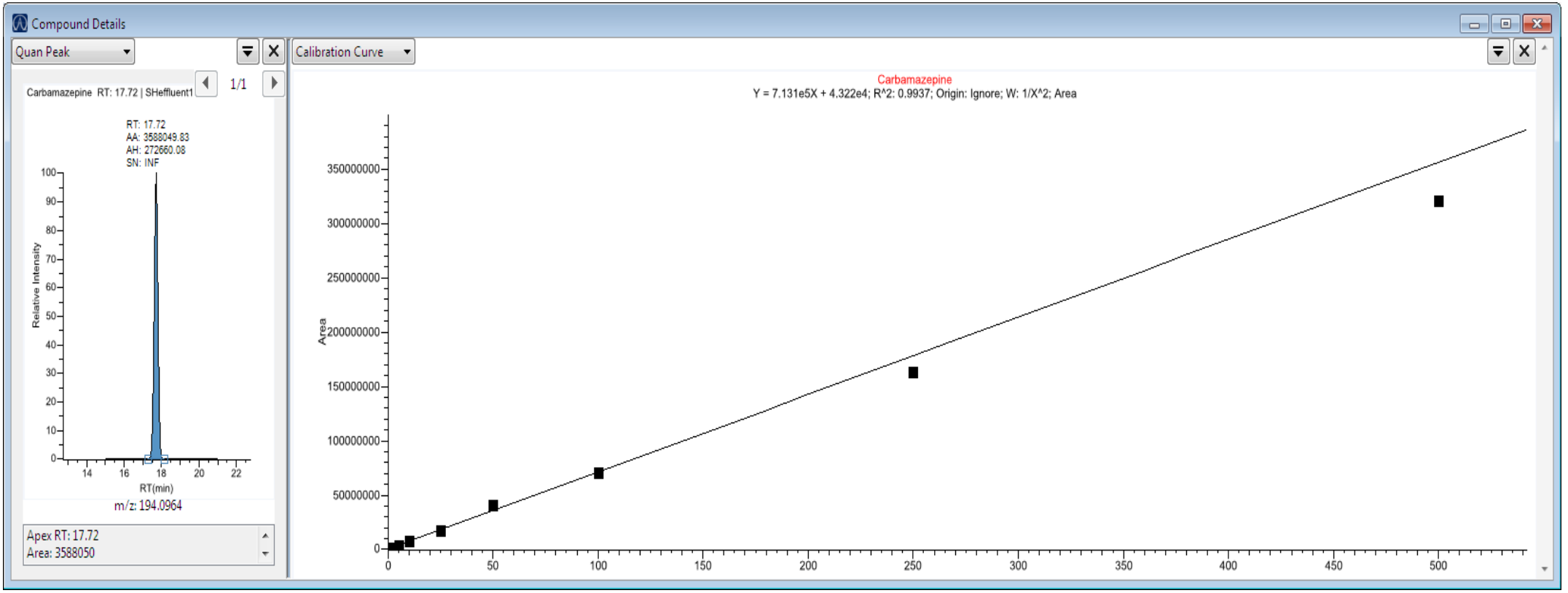
Calibration curve and chromatogram for atenolol



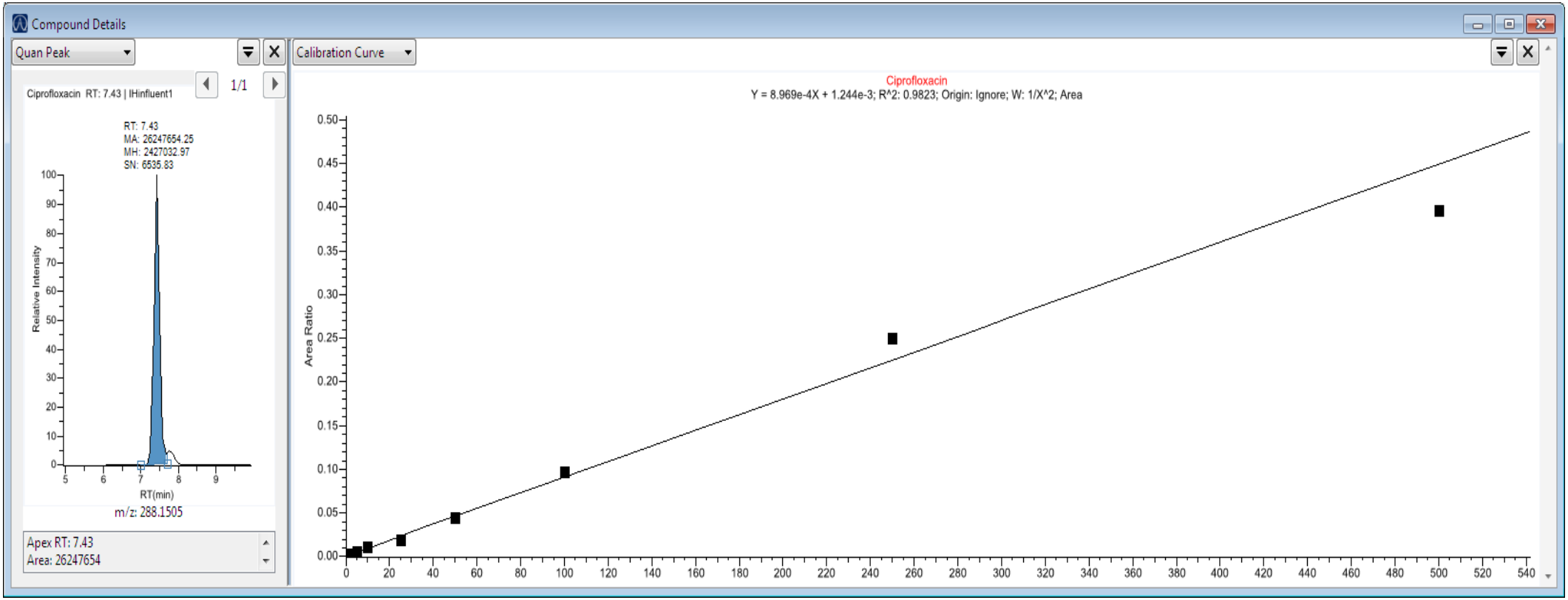
Calibration curve and chromatogram for bezafibrate



Calibration curve and chromatogram for carbamazepine



Calibration curve and chromatogram for ciprofloxacin



Decreasing concentrations of selected pharmaceuticals in aerobic and anaerobic batches

		Value expressed as % peak area of T_0					
		Sample	Naproxen	Ibuprofen	Sulphamethoxazole	Paracetamol	
Hamed1	Mix-0A SRT	MIXWO0asrt	100.00	100.00	100.00	100.00	
Hamed2	Mix-1A SRT	MIXWO1asrt	100.00	98.23	100.00	87.21	
Hamed3	Mix-2A SRT	MIXWO2asrt	99.00	93.49	98.01	67.41	
Hamed4	Mix-5A SRT	MIXWO5asrt	95.66	89.66	94.23	44.56	
Hamed5	Mix-8A SRT	MIXWO8asrt	88.16	84.50	94.00	30.77	
Hamed6	Mix-11A SRT	MIXWO11asrt	76.92	79.12	94.89	10.90	Aerobic digestion
Hamed7	Mix-15A SRT	MIXWO15asrt	68.16	66.58	93.10	0.00	
Hamed8	Mix-0B SRT	MIXWO0bsrt	100.00	100.00	100.00	100.00	
Hamed9	Mix-1B SRT	MIXWO1bsrt	99.00	97.92	99.23	91.09	
Hamed10	Mix-2B SRT	MIXWO2bsrt	98.19	97.56	99.20	75.13	
Hamed11	Mix-5B SRT	MIXWO5bsrt	97.00	94.51	98.26	54.79	
Hamed12	Mix-8B SRT	MIXWO8bsrt	92.04	86.33	97.32	35.96	
Hamed13	Mix-11B SRT	MIXWO11bsrt	79.23	79.01	96.59	17.26	
Hamed14	Mix-15B SRT	MIXWO15bsrt	63.92	73.29	96.00	4.47	
Hamed15	AD B1-0 SRT	AD B1 OD	100.00	100.00	100.00	100.00	
Hamed16	AD B1-2 SRT	AD B1 2D	91.16	98.01	59.73	49.16	
Hamed17	AD B1-4 SRT	AD B1 4D	90.01	88.01	33.23	40.09	
Hamed18	AD B1-6 SRT	AD B1 6D	87.16	83.20	17.09	33.64	
Hamed19	AD B1-8 SRT	AD B1 8D	77.83	81.38	5.45	26.50	
Hamed20	AD B1-10 SRT	AD B1 1OD	74.17	80.13	0.00	18.00	Anaerobic digestion
Hamed21	AD B2-0 SRT	AD B2 OD	100.00	100.00	100.00	100.00	
Hamed22	AD B2-2 SRT	AD B2 2D	87.03	100.00	75.21	63.91	
Hamed23	AD B2-4 SRT	AD B2 4D	87.88	90.97	43.59	51.25	
Hamed24	AD B2-6 SRT	AD B2 6D	85.05	88.79	21.04	46.06	
Hamed25	AD B2-8 SRT	AD B2 8D	81.64	84.00	10.42	33.50	
Hamed26	AD B2-10 SRT	AD B2 10D	77.00	79.61	3.30	23.05	